# ORAL LESIONS IN HIV SERO POSITIVE PEDIATRIC PATIENTS AND ISOLATION AND SPECIATION OF ORAL CANDIDA

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

ORAL AND MAXILLOFACIAL PATHOLOGY

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# CERTIFICATE

This is to certify that this dissertation titled **"ORAL LESIONS IN HIV SEROPOSITIVE PEADIATRIC PATIENTS AND ISOLATION AND SPECIATION OF ORAL CANDIDA"** is a bonafide record of work done under our guidance during the study period between 2003-2006.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the Degree of **MASTER OF DENTAL SURGERY - ORAL AND MAXILLOFACIAL PATHOLOGY, BRANCH IV.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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# CONTENTS

# *Introduction*

The world has never seen a comparable complex public health hazard as that which it is currently experiencing in dealing with the effects of Human immunodeficiency virus infection (HIV) /Acquired immunodeficiency syndrome (AIDS) pandemic. The impact of HIV disease on children is not limited to their increasing rates of infection, but also to the fact that in 10 years time over 40 million children are expected to be orphaned as a result of AIDS[45]. HIV infection is posing a life long threat to children, with over 500,000 infected as of 2005[138,139].

Three important factors influencing epidemiology of pediatric HIV infection are: (1) The prevalence of HIV among women in childbearing age (2) The availability and effective use of antiretroviral and prophylactic therapy for children (3) Institution of antiretroviral medications during pregnancy. Almost half of all infected individuals are women of childbearing age[25, 45]. Little or no antiretroviral medications are available for these women. Also children in developing countries do not have access to life-saving drugs. In contrast the picture is very different in the developed countries as testing pregnant women for HIV infection and institution of antiretroviral medications during pregnancy for HIV-infected women are becoming common practices[25, 45].

Vertically acquired HIV infection accounts for 85% of all reported pediatric cases world wide[25, 45]. The vertical transmission can occur in several ways: maternal bodily fluids during labour and delivery, breast feeding and through the placenta early in gestation[25, 45]. These are multiple determinants of transmission and include maternal viral load, viral phenotype, obstetric factors and maternal immune response[25, 45]

Following the initial report in 1982 of HIV infection in children it became evident that the clinical characteristics of HIV infection in children were different from those of

adults. In 1987, CDC published a classification system for children infected with HIV. The classification system categorized clinical manifestation of HIV infection in children based on limited data available early in epidemic[19]. New knowledge about the progression of HIV disease among children warranted revision of the 1987 classification system to reflect the disease process better. In 1994, CDC convened a working group of public health service to revise of the pediatric HIV infection classification system. They system classified pediatric HIV infection into exclusive categories based on three parameters a) Infection status b) Clinical status and c) Immunologic status[19].

In children, individual sign and symptoms are often non-specific. Unlike adults, vertically infected children have an immature immune system and consequently present with a shorter incubation period and more rapid and fulminate disease process (Rosenberg and Fauci, 1994). Clinical presentation of orofacial lesions in patients with HIV infection is strongly associated with immune suppression[25, 45].

Due to the profound immunosuppression HIV infected pediatric patients are more susceptible to opportunistic infections particularly fungal infections like candidiasis, cryptococcoidosis, histoplasmosis and aspergillosis [110]. Of these candidiasis is the most common infection and occurs in 63% of the pediatric population[56].

Pseudomembranous candidiasis is the most common type (Valdez et al and Santos et al) followed by erythematous type and angular chelitis[45]. Candidia albicans, Candidia glabrata, Candidia tropicalis, Candidia dubliniensis and Candidia krusei have been associated with HIV infection [15, 54, 66, 111]. The conventional methods available to isolate and subtype them involve significant technical expertise and are time consuming. Newer technique like CHROM agar help to identify different species[15, 66].

Oral lesions of HIV in adults are relatively well described in the literature but comparative data on pediatric HIV infection is limited particularly in developing countries like India [25, 44].

# *Aims & Objective*

**HYPOTHESIS:**

1. Oral lesions are a major feature of HIV seropositive pediatric patients.
2. Candidiasis in HIV infection is due to Candidia albicans.

**AIMS AND OBJECTIVES:**

1. To identify and document oral lesions in HIV seropositive pediatric patients.
2. To isolate candida from both symptomatic and asymptomatic HIV seropositive pediatric patients
3. To speciate and quantitate candidia in both symptomatic and asymptomatic HIV seropositive pediatric patients.

# *Review of Literature*

**"Pediatrics** is a branch of medicine which is concerned with the health of infants, children and adolescents, their growth and development, and their opportunity to achieve full potential as adults." By Richard E.Behrman [141]

**Pediatric dentistry** definition was changed in July 1994 by the American dental association council, as " It is an age defined speciality that provides both primary and speciality comprehensive preventive and therapeutic oral health care for infants and children through adolescence, including those with special health care needs"[140]

## PEDIATRIC HIV INFECTION

Over the past decade, the global pandemic of HIV has spread to every continent, with nearly 13million cases worldwide. Of these cases 1 million have occurred in children. Since the first childhood case was reported in 1983 the number of children infected with the HIV virus has grown almost exponentially. Pathogenesis of pediatric HIV is different from that of adult and it depends on multiple factors which include high viral load and lower CD4 cell count which reflect the maternal diseases[45].

**Geographical distribution:**

**Kamal H and Rathore MH (1997)**[63] pediatric HIV infection continues to be a challenge for the medical community. It is an evolving entity and our knowledge of HIV is increasing rapidly with newer finding. Florida is the second highest number of pediatric AIDS cases among developed countries.

**Grant AD and Cock KM (2001)**[49] the epidemiology and burden of HIV in the developing world are different than the HIV status in developed countries. Homosexual

transmission is common route of transmission of HIV in developed countries were as vertical transmission (children) and heterosexual transmission is the common mode of transmission seen in the developing countries. HIV 2 infection is the common type of HIV seen in developed countries.

**Rothrock SG (2004)**[113] over the past decade, the global pandemic HIV has spread to every continent, with nearly 13 million cases world wide. Classification system in child is based on the CD4 count level and the diagnosis is made only when the children is more than 12 months of age. The common clinical presentation varies according to the endemic areas.

**Mahal A and Rao B (2005)**[82] India now appears on the brink of significant AIDS epidemic. More consideration is given to the clinical aspect of the diseases, but one of the major cause of spread of HIV infection in India is adverse household level economic status. They concluded that household-level economic status has an impact on HIV infection in developing countries like India.

**Global AIDS Epidemic/ UNAIDS (2005)**[138] More than 1,900 children worldwide are infected with HIV each day. In 2005, an estimated 4.8 million people (range: 4.2-6.3 million) became newly infected with HIV -- 630,000 of them were children (range: 570,000--740,000). The vast majority of them were infected antepartum (20% before childbirth; during pregnancy); intrapartum (40% during childbirth), or breastfeeding (40%). Today, some 37.8 million people (range: 34.6-42.3 million) are living with HIV -- 2.1 million (range: 1.9-2.5 million) of them are children under the age of 15. In 2003, 2.9 million people (range: 2.6-3.3 million) died of AIDS -- 490,000 (range: 440,000-580,000) were children. This amounts to approximately 1,350 AIDS deaths in children per day.

Worldwide, over 20 million people have died from AIDS since the first cases were identified in 1981. There are children orphaned by AIDS in almost every country of the world. Children orphaned by AIDS range in age from a few days or months old to 18 years of age. In countries with low-level and concentrated epidemics, it is impossible to reliably estimate the number of children orphaned by AIDS, or to determine what percentage they represent of all orphans. By 2010, this number is expected to climb to more than 18 million.

**NACO India (2005)[139]** Out of 1111608 HIV infected patients in our country, about 4854 are children and they are between the age group of 0-14 years.

**Classification:**

With the identification of causative agent of AIDS a broad spectrum of clinical manifestations has been attributed to infection with HIV. With the expectation of the CDC (Center for Diseases Control) surveillance definition for AIDS, no standard definition for other manifestations of HIV infection has been developed for children[19,29].

First classification was published in 1987 based on the clinical presentation this classification system has been developed primarily to categorize clinical presentations in adult patients and may not be entirely applicable to infants and children. With new knowledge about the progression of HIV disease among children warranted revision of the 1987 classification system to better reflect the disease process. New classification is based on infection status, clinical status and immunologic status of the HIV infected children[19, 29].

**CDC 1987**[29] Framed the first classification for HIV infected pediatric patients in to three groups which were based entirely on the clinical presentation.

**CDC 1994**[19] with the additional knowledge about the progression of HIV diseases among children, a new classification was framed which categories the HIV infected children according to infection status, clinical status and immunological status.

**Marnino LM, Tova PA, Gabiano C *et al*(2000)**[84] They compared the CDC pediatric classification system with the long time course of prenatal HIV type I infection, the studied 366 prenatal patients starting from birth at least every three months for mortality, manifestations etc. They observed that clinical status was comparable with the CDC classification but the immune status of infants and patients in categories B showed better prognosis than other categories.

**Age distribution:**

**Kamal H and Rathore MH (1997)**[63] Most of the children affected are between the age group of 4-7 years.

**NACO India (2005)**[139] Out of 1111608 HIV infected patients in our country, about 4854 are children and they are between the age group of 0-14 years.

**Pathogenesis:**

**Kamal H and Rathore MH (1997)**[63] pediatric HIV infection continues to be a challenge for the medical community. HIV infection progress in two general patterns in HIV infected patients; first mode is rapid progression when severe immunodeficiency develops in the first year of life in 15-20% of children. The remaining 75% to 80% have a form of disease that progress slowly and more similar to that of adults.

**Church JA (2000)**[26] HIV infection in children is different from that of an adult; it has an bimodal pattern of progression and there is no specific CD4 that defines the condition unlike for patients over 13 years of age. HIV test can not do for children with in

18months of life since most of the children born to HIV infected mothers will be positive till one year of life. They are more pronounced to bacterial infections than adults.

**Banerjee U (2005)**[4] Several analyses of data from pediatric registries and retrospective studies have suggested that the progression of the acquired immunodeficiency syndrome (AIDS) follows two patterns in children who are infected with the human immunodeficiency virus by their mothers. In the first year of life, severe immunodeficiency develops and the other pattern is a form of disease that progresses more slowly and is probably similar to that observed in adults. They studied the influence of clinical and biologic characteristics of HIV-infected mothers at the time of delivery on the progression of disease and death in 162 infected children. They observed that infants whose HIV infection is maternally acquired, the rate of disease progression varies directly with the severity of the disease in the mother at the time of delivery.

**Godbole S and Mehendale S (2005)**[46] since the first report of HIV infection in India in 1986, the virus has spread all over the country although there are geographic variations. It is estimated that 5.1million people are infected with HIV. Both HIV serotypes 1 and 2 exist in India and HIV 1 C is the commonest subtype reported.

**Route of transmission and prevention:**

Most children who are infected with HIV acquire the virus through mother-to-child transmission. Without any intervention, overall mother-to-child transmission (MTCT) rates range between 25% and 45% in developing countries, and between 16% and 20% in Europe and North America[114]. The prevalence and duration of breast-feeding, prematurity, and the maternal viral load, as well as the different methods used to classify infant infection status and calculate transmission rates, may account for the differences in

reported perinatal HIV-1 transmission rates around the world. MTCT can occur during pregnancy – in utero or labour and delivery – intrapartum, and through breast-feeding – postpartum[114]. The absolute risk for in utero transmission is estimated to be approximately 5% to 6%, and for intrapartum transmission, approximately 13% to 18%. In many developing countries where breast-feeding is almost universally practiced, postpartum transmission through HIV-1 contaminated breast milk has been widely documented[114].

**Rutherford GW, Oliva GE, Grossman M *et al*(1987)**[115] the transmission of HIV infection from infected mothers to infants either utero or perinatally has been established. The rate of transmission in developed and developing countries various. Preinatal transmission can be avoided by educating the mother and with the availability of ART drugs.

**Wara DW (1995)**[135] approximately 2% of people with AIDS in United States are children. More than 90% of children acquire HIV infection through vertical transmission. Administration of antiretroviral therapy during the delivery or 6week after delivery is the major way to prevent HIV infection in children.

**Kamal H and Rathore MH (1997)**[63] pediatric HIV infection continues to be a challenge for the medical community. The predominant mode of transmission is through vertical transmission.

**Raeza Dm (1999)**[108] compared the HIV infected children whose mothers were treated with antiretroviral drug with children whose mother were not treated with antiretroviral drugs. They screened 216 Italian patients and estimated the probability of

developing sever diseases or sever immune suppression. They found that 37.2% of first group had low chance of developing diseases than the other group of patients.

**Jain A and Davis MM (2001)**[60] 95% of HIV infected children are living in developing countries. The major mode of transmission in them is vertical transmission. This can be prevented by administration of antiretroviral drugs intravenously during delivery or 6week after birth.

**Hermione EG (2002)**[53] 40 million people were infected with HIV infection and majority were in South Africa. The common mode of transmission was vertical transmission and in breast fed children it is estimated to be 25-45% when compared to non breast fed children.

**Black E and Robert CG (2002)**[13] Breast feeding continues to be the norm in low income countries, but the period of exclusive breast feeding after birth is often short. WHO recommended 6 month of breast feeding is needed to protect the child from endemic diseases but with the recognition that HIV can be transmitted from infected mothers to their infants through breast milk. In order to prevent HIV transmission affordable and safe infant formula food, withholding breast milk is recommended.

**Steinbrook R (2002)**[121] most of the children who are infected with HIV infection were through perinatal transmission. With the admistration of Antiretroviral drug, the perinatal transmission can be prevented.

**Godbole S and Mehendale S (2005)**[46] Mother to infants transmission is 36-48% in India. It has been reported that 63% of multi-transposed thalassaemic HIV infected children became symptomatic in infancy with a 9% fatality rate with in 14 months of age.

Antiretroviral therapy is given to the pregnant women from the last 6weeks and delivered by elective caesarians section.

**Investigations:**

**Clogg D, Delage G, Halperin S (1993)**[27] when the patient develop symptoms like diarrhea, unknown fever, lymphadenopathy, hepatomegaly, the child should be tested for HIV infections and it is usually done for those patients who are more than 18 months of age since children born to HIV infected mothers will be positive if tested within one year of age; so there are possibility of false positive results.

**Villalobos TI (1999)**[131] significant changes have occurred in management of HIV infection in children since the first case was reported in 1983. But it still continues to be one of the leading causes of death in children. It progresses in bimodal way and the major mode of transmission is by vertical transmission. Antibody testing in infant is usually done after 18months of age. Perinatally admistration of antiretroviral drug will help to reduce the transmission of HIV infection from the mother to child.

**Church JA (2000)**[26] HIV infection in children is different form that of an adult; it has an bimodal pattern of progression, there is no specific CD4 that defines the condition, in patients over 13 years of age. HIV test can not be done for children with in 18months of life since most of the children born to HIV infected mother will be positive till one year of life. They are more pronounced to bacterial infections than adults.

**Weinberg A and Pott GB (2003)**[136] they correlated CD4 count, cell mediated immunity in 12 patients who were on antiretroviral therapy. They found that there is an significant correlation between the degree of CD4 count and the effect of antiretroviral therapy.

**Ramesh SP (2005)**[104] Even though the prognosis of HIV is decided by plasma virus load set point, the rate of decline in CD4 count, increase in plasma virus load and the HIV associated opportunistic infections. HIV specific CD4 helper response and HIV-specific CTL responses clearly emerge as the most important host factors that may decide the rate of disease prognosis.

## ORAL LESION IN HIV PEDIATRIC

Infection with HIV results in profound immunosuppression and render the host susceptible to the development of various opportunistic infections and neoplasms[91]. Compared with adult, the progression of HIV infection is more rapid and severe in infants and children due to the ongoing development of different organ system and an immature immune system that is less resistant to infection [22]. Oral lesions are frequently the first symptoms in HIV infected children. Early detection of HIV related oral lesions could be used to diagnose HIV infection, elucidate progression of the disease, predict immune status, and provide timely therapeutic intervention[107, 91]. Using the frame work of EC-Clearinghouse and WHO a consensus classification of oral lesion associated with pediatric HIV infected patients was formed in 1994 [97]

**Ferguson FS, Berenten B, Nachman S (1995)**[35] studied oral manifestations in 58 HIV infected patients and found that pseudomembraneous candidiasis was observed in 29% of cases, parotid gland swelling is seen in 23.5% of cases, gingival inflammation was seen in 5.2% of cases and hairy leukoplakia was seen in 3.2% of cases. They found that incidence of dental caries was more in patients with decreased immune status.

**Leggott PJ (1995)**[74] stated that in adults, oral lesions are frequently noted among HIV infected children. The most common fungal infection observed was oral candidiasis seen in 11%-72% in symptomatic patients and 11%-20% in asymptomatic patients. Presence of oral candidiasis indicate the immunosuppression state of the children. Other fungal infections associated with HIV infected patients are crytococcosis, histoplasmosis, geotrichosis and aspergillosis. The common viral lesion seen in them are herpes simplex

virus (24%), herpes zoster and oral hairy leukoplakia caused by Epstein-bar virus. The neoplasms among the children are kaposis sarcoma, non- Hodgkins lymphoma which is less prevalent when compared to adult HIV infected patients.

**Broder HL, Catalanotto FA, Reisine S *et al*(1996)**[17] did a longitudinal study to examine the compliance rate among HIV positive patients; HIV negative patients and HIV- positive siblings. 105 patients were screened. The major complaint in them was dental caries, oral pain and gingivitis. Each patient was followed for every 6 months. They found that the children with HIV and their siblings have significantly lower compliance than the regular patients. This is indicated the need for improving our understanding to care for HIV infected children.

**Chigurupati R, Raghavan SS, Deborah A (1996)**[25] reviewed the common oral manifestations observed in HIV infection pediatric patients. Oral candidiasis, herpetic eruptions, oral hairy leukoplakia, oral ulcers, linear gingival erythema, cervical lymphadenopathy were the common oral lesions seen in HIV infected children.

**Howell RB, Jandinski JJ, Palumbo P *et al*(1996)**[57] studied the oral manifestation in 60 HIV positive children in USA and correlated the prevalence of oral lesion with the CD4 count. Oral candidiasis in 32% of cases, conventional gingivitis in 45% of patients, linear gingival erythema in 38% of cases and periodontitis was observed in 8% of cases. They observed a significant difference between the prevalence of oral candidiasis, healthy gingival and periodontitis and the CD4 count but there was no relationship between the linear gingival erythema, conventional gingivitis and the CD4 count. They concluded that with the decline in the CD4 count they is an definite increase in prevalence and severity of candidiasis and gingivitis.

**Ramos F, Hiltons JS, Greenspan JS *et al*(1996)**[107] retrospective study on 492 perinatally infected HIV pediatric patients in California. They were screened in order to find the prevalence of oral facial soft tissue manifestations in HIV infected children and normal children and also to identify the risk factor for occurrence of oral lesions. 91 HIV infected children and 185 HIV negative children were taken. Oropharyngeal candidiasis was seen in 67%, parotid gland enlargement was seen in 19%, herpes simplex virus in 3% of patients. They conclude that the occurrence of oropharyngeal candidias in children is due to presence of vaginal candidiais during delivery and this lesion could be used as one of the indicators for risk factor determination in HIV infected children.

**Toro AD, Berkowitz R, Meyerowitz C *et al*(1996)**[127] screened 28 HIV infected patients in New York to find the prevalence of oral lesion in them. Pseudomembraneous candidiasis was seen in 27.8% of patients, ulcer in 7.1%, delayed tooth development in 7.1% of cases, parotid swelling in 5.5% and petechiae was seen in 39.3% of cases.

**Luciance RR, Peacock CS, Hounnou A *et al*(1998)**[77] screened 40 HIV positive Brazilian pediatric patients to find the prevalence of oral lesion in them. Cervical lymphadenopathy was the most common lesion observed in them followed by pseudomembraneous candidiasis in 22% of patients, angular chelitis in 9.8% of cases, erythematous candidiasis in 4.9% of cases and ulcer in 4.9% of patients.

**Reddy BV, Ranganathan K, Kumarasamy N *et al*(1998)**[109] screened 13 HIV positive pediatric patients in Chennai. The prevalent oral lesions observed were Pseudomembranous candidiasis, erythematous candidiasis, chronic gingivitis, apthous ulcers, parotitis, lymphadenopathy. The common systemic lesions were pulmonary

tuberculosis, herpes zoster, lymphadenopathy, lower respiratory tract infections and staphylococcal skin infections.

**Vieria AR, Riberiro LP, Modesto AC *et al*(1998)**[130] studied the gingival health in 43 HIV positive patients in Brazil. They found that the severity of gingival diseases is more in patients with severe immunosuppression state and had high prevalence of dental caries.

**Arendorf T and Holmes H (2000)**[3] studied the various oral manifestation seen in HIV infected adult patients. They are different in the developed and developing countries but this data is not available for HIV infected pediatric patients. Malnutrition and nutritional deficiencies, especially protein energy malnutrition is common in patients infected with HIV but pneumocystis carinii is common in the developed countries.

**Baransch A, Safford M, Catalanotto FA *et al*(2000)**[5] did a longitudinal study for two years in New York, to compare the various oral manifestations in HIV positive and HIV negative patients. 104 HIV positive patients were screened. Pseudomembraneous candidiasis was seen in 19% of patients, atrophic candidiasis was seen in 9%, linear gingival erythema in 22%, median rhomboid glossitis in 12%, conventional gingivitis in 7%, necrotizing periodontitis in 3.8% and hairy leukoplakia in 2%. They concluded that oral candidiais was the common oral lesion observed in HIV infected children and it can be used to asses the disease progression and immune status but it is a poor indicator for mortality.

**Dos LD, Fernanda G, Riberiro LP *et al*(2000)**[30] screened 80 HIV infected Brazilian pediatric patients in order to correlate the oral lesions with the degree of immunosuppression. They observed 38% of oral lesions in patients with low CD4 count.

The common oral lesion observed in them was oral candidiasis in 22.5% patients, 17.5 % had gingivitis, parotid gland enlargement was observed in 8.8% of cases, herpes simplex was seen in 1.3% of cases and hairy leukoplakia was observed in 1.3% of cases.

**Eldridge K and Gallagher JE (2000)**[33] screened 30 HIV positive children and 20 HIV negative children in London to evaluate dental caries prevalence in these groups. They observed that the prevalence of dental caries in positive children is 60% more than non infected children and this is because of unfavorable dental behaviors like consumption of extrinsic sugar in night, use of sugar based medications, low fluoride intake, late commencement of tooth brushing and poor dental attendance.

**Flanagan MA, Barasch A, Koenigsberg SR *et al*(2000)**[39] studied the prevalence of oral manifestation in 38 HIV infected pediatric patients treated with antiretroviral therapy. Oral candidiaisis in 36 % of cases, conventional gingivitis in 50% of patients, linear gingival erythema in 32% of case, median rhomboid glossitis in 8%, parotitis in 3% of cases, herpes labialis and hepetic gingivitis in 3% of cases are the common lesions observed in their study. They concluded that there is no significant difference in prevalence of oral lesion in patients with HAART and without HAART.

**Gelbier M, Lucas VS, Zervous NE *et al*(2000)**[44] studied the prevalence of dental caries and gingival diseases in 35 HIV positive patients in London. Dental caries was more common in deciduous tooth than in permanent tooth and 60% of patient had gingivitis. Other common oral lesions observed in their study were pseudomembraneous candidiasis, erythematous candidiasis and one case of hyperplastic candidiasis.

**Gomez FJ, Petru JF, Hilton AJ *et al*(2000)**[47] did a retrospective study in 40 perinatally HIV infected children in California to describe the incidence and prevalence

of oral manifestations in them. They found that the incidence of oral candidiasis was 43% and was positively associated with the low CD4 count and occurrence of plaque, and they observed that in patients with severe immunosuppression there was delayed tooth eruption and high incidence of dental caries.

**Hicks MJ, Flaitz CM, Carter AB *et al*(2000)**[55] did a descriptive longitudinal clinical study in 73 HIV infected children to determine caries in primary and permanent dentition and compared it with CD4 immune status. They observed that primary caries increases with decrease in CD4 count and the caries free state decrease with increase in age and decrease in immune status. This is due to admistration of high carbohydrate diet in HIV infection patients in order to improve their health status and the use of sugar rich medication.

**Konzinetz CA, Carter B, Simon C *et al*(2000)**[70] assessed the prevalence of oral lesion in 73 HIV positive pediatric patients in Brazil. The predominant lesion observed was cervical lymphadenopathy seen in 51% of cases, hairy leukoplakia in 3% of cases, parotid gland enlargement was seen in 12% of cases, oral candidiasis was seen in 41% of cases, and xerostomia was seen in 44% of cases. They also observed that the severity of oral lesion increases with the decreases in immune status.

**Oralloda WS and Talmitis NI (2000)**[95] screened 762 HIV positive pediatric patients for oral lesions. The common oral lesion observed were 18.42% oral candidiasis, 28.9% angular cheilitis, conventional gingivitis in 13.1%, herpes simplex in 5.2%, hairy leukoplakia, recurrent ulcers and condyloma in 2.63%. Enamel hypoplasia was seen in 23.6% of cases.

**Schoen DH, Murray PA, Nelson E *et al*(2000)**[117] A longitudinal study was done to compare the periodontal diseases in 68 HIV infected and 53 household peers. They found that occurrence of linear gingival erythema is more common in the HIV positive group.

**Tofsky N, Nelson EM, Lopez RN *et al*(2000)**[126] studied the prevalence of dental caries between 104 HIV positive and 67 HIV negative pediatric patients in New York population. They found that the incidence of caries is 17% higher than the negative group and the incidence of caries is more in patients between the age group of 6-11years and in deciduous teeth. This is due to unfavorable dental habits and also due to delay in eruption of permanent teeth.

**Flatiz C, Wullbrands B, Sexton J *et al*(2001)**[36] studied the prevalence of oral lesions in 173 HIV infected Romanian pediatric patients. The most common oral lesion observed was oral candidiasis in 29 % of cases, ulcers in 15% of cases, parotid gland enlargement in 9% of cases, necrotizing ulcerative gingivitis in 5% of cases, linear gingival erythema in 4% of cases, labial molluscum contagiosum in 3% of cases, oral wart in 2% of cases, hairy leukoplakia in 2% of cases and herpes zoster in 1% of patients. Dental caries was present in 46% of cases.

**Hauk MJ, Moss ME, Weinberg GA *et al*(2001)**[51] assessed the relationship between delayed tooth eruption and the progression of pediatric HIV infections to AIDS in 70 perinatally infected children in New York. They did an analysis between the dental age and chronological age and found that the clinical symptom status was strongly associated with the delayed tooth eruption in 60% of cases but there was no significant

association between CD4 level and delayed tooth eruption. They concluded that the delayed tooth eruption is due to the severity of symptoms and not due to CD4 depletion.

**Kozinetz CA, Matusa R, Cazacu A *et al*(2001)**[71] did a cross-sectional study in Romania to observe the predominant oral lesion seen in 762 HIV positive patients. Lymphadenopathy 100%, otitis media 97%, pneumonia 50%, candidiasis 68%, lymphoid interstitial pneumonia 50%, recurrent herpetic eruptions 50%, recurrent bacterial infection 97%, kaposis sarcoma in 2 cases.

**Birnbaum W, Hodgson TA, Reichart PA *et al*(2002)**[12] studied the oral manifestation of HIV infection has been considered to be of value in assessing diseases progression in the developed country, however, the potential use of oral lesions as prognostic markers in developing countries is yet to be investigated this is due to less availability of dates from the developing countries. With the introduction of HARRT therapy a vast change in prevalence of oral lesions. There is regression in the occurrence of oral candidiasis, kaposis sarcomas and oral hairy leucoplakia. However there is no change in the prevalence of oral condylomata and herpes simplex infections. Further research should be conducted in developing countries to know the real status of HIV in the world.

**Gomez FR (2002)**[48] stated that oral lesions are early and common clinical indicators and progression markers of HIV infection. The common oral lesions are oral candidiasis, of which pseudomembranous candidiasis was common followed by erythematous candidiasis and hyperplastic candidiasis. The prevalence of other lesions like parotid gland enlargement is 10-30% and herpes simplex is 1.7-24%. Periodontal lesions commonly seen in HIV infected children are linear gingival erythema, necrotizing

ulcerative gingivitis, necrotizing ulcerative periodontitis and necrotizing ulcerative stomatitis. Dental caries is common in these patients due to drugs and due to immunosuppression. Rare lesions seen in the pediatric group are oral hairy leukoplakia, kaposis sarcoma and non-Hodgkins lymphoma.

**Patton LL, Phelan JA, Gomez FJ *et al*(2002)**[97] reviewed the common oral lesions between HIV positive adults and pediatric patients. The common lesion in both children and adults are Non-CNS opportunistic infections, Chronic mucocutaneous candidiasis, Neural abnormalities, Chronic fever and diarrhea, Diffuse adenopathy, Hepatomegaly, Chronic eczema, renal diseases and cardiomyopathy. The lesion common in childrens than in adults are recurrent bacterial infections, chronic interstitial pneumonia, acquired microcephaly and failure to thrive.

**Teo CG (2002)**[124] stated that in the global terms, the two principal viral associated oral diseases in HIV infected patients are oral hairy leukoplakia and kaposis sarcoma. Both are prominent in America, Europe and Australia but appear to be less among patients in south Asia and Far East. In Africa AIDS associated kaposis sarcoma is commonly encountered but the prevalence figure for OHL varies considerably.

**Mark WK (2003)**[83] did a cross-sectional study from the year 1990-1994 in 158 patients, the common oral lesions observed in his study were, oral candidiasis in 117 patients, herpetic eruptions in 20 patients, apthous ulcer in 23 patients, parotid enlargement in 9 patients. He stated that variety of infectious, neoplastic and inflammatory conditions can produce cutaneous or oral lesions in HIV infected patients. Oral hairy leukoplakia, bacillary angiomatosis, kaposis sarcoma are common lesion observed exclusively in HIV infected patients, herpes simplex virus, varicella-zoster

virus, molluscum contagiosum, candidiaisis, scabies dermatitis, apthous ulcers, periodontal diseases, staphylococcal infections are the lesions that occur with severity in HIV infection.

**Okunseri C, Badner V, Wiznia A *et al* (2003)**[94] screened 102 HIV infected pediatric patients in New York and compared with the CD4 levels. 69% had oral lesions and 31% were disease free. The common oral lesions observed were conventional gingivitis 20.6%, pseudomembranous candidiasis 2.9%, depapillation of tongue 13.7%, median rhomboid glossitis 1%, bilateral parotid gland enlargement 2%, lymphadenopathy 1%, linear gingival errythema in 2.9% and dental caries in 2.9%. Patients with low CD4 count had more gingival lesions than the other two groups, which was statistically significant. They concluded that children with low CD4 count had more oral lesions.

**Pongsiriwet S, Lamaroon A, Kanjanavanit S *et al*(2003)**[102] described the prevalence of oral lesions and dental caries in 40 HIV infected Thailand pediatric patients. Oral candidiasis was the most common oral lesion observed in 45% of cases and the Pseudomembraneous candidiasis was the common variant of candidiasis observed in 32.5% of cases, erythematous candidiasis in 25 % of patients, angular cheilitis in 4 patients, linear gingival erythema in 20% of cases and recurrent aphthous ulcer was seen in 5% of cases. Dental caries was seen more in the patients between the age group of 6-9 years.

**Reichart PA (2003)**[110] said that the most common fungal infection in HIV infected patients is oral candidiasis and is strongly associated with HIV infection. It occurs in 9-90% of cases and the most common variants are pseudomembraneous candidiasis and erythematous candidiasis. Common oral bacterial lesions seen in HIV

infection are periodontal lesions like linear gingival erythema, necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis. Kaposis sarcoma predominately occurs on the palate and tongue and is observed in 60% of adult HIV infected patients and rare in HIV infected pediatric group. With the introduction of HARRT therapy the oral manifestations such as oral candidiasis, gingivo-periodontitis and kaposis sarcoma are rarely seen.

**Nadioo S and Chikte U *et al*(2004)**[91] studied the various oral manifestation in 169 HIV infected hospitalized patients in South Africa and compared them with the institutional population. They found pseudomembraneous candidiasis in 50%, erythematous candidiasis in 29%, angular chelitis in 10%, hairy leukoplakia in 1 case, cervical lymphadenopathy in 18%, and parotid gland enlargement in 7 cases. The prevalence of these lesions are four times more in hospitalized patients than in institutional patients but the prevalence of oral ulcer is more in institutional patients.

**Glick M (2005)**[45] Although many clinical features are common to both adult and children, lesions like failure to thrive, abnormal growth rate, lymphoid interstitial pneumonia, invasive and recurrent bacterial infections, parotitis are pronounced only among the pediatric population. The common malignancies in children are non-Hodgkins lymphoma and leiomyosarcoma. Kaposis sarcoma is common in adults but it is rare in children. The common oral lesions are oral candidiasis, parotid gland enlargement, lymphadenopathy. The prevalence of orofacial manifestation differs among geographic regions and this is due to the level of medical care provided to the HIV infected children.

## SYSTEMIC LESIONS AND ITS PREVALENCE IN HIV PEDIATRIC PATIENTS

HIV infection has become one of the greatest pandemics ever. Young infants may present with sign and symptoms of HIV infection that strongly suggest intrauterine infection; other children may show no outward signs of HIV for more than 10 years [26]. The common clinical presentation seen in HIV infected pediatric patients are chronic diarrhea, delayed development, failure to thrive, hepatomegaly, lymphadenopathy, lymphomas,opportunistic infections, recurrent otitis media, sinusitis, pneumonia, severe molluscum contagiosum or condylomas, splenomagaly, herpetic infection and other viral infections[26]. The clinical presentation various depending upon the severity of immunosuppression[2, 91, 57].

**Theuer CP (1989)**[125] Tuberculosis is a frequent complication of human immunodeficiency virus (HIV)-induced immunosuppression. The diagnosis of extrapulmonary tuberculosis in patients with evidence of HIV infection qualifies as a criterion of the acquired immunodeficiency syndrome. Demographic characteristics of patients with tuberculosis and HIV infection vary by region and reflect the degree to which patients with Mycobacterium tuberculosis infection adopt behaviors that put them at risk for HIV infection. Treatment is effective for tuberculosis in HIV-seropositive patients, Isoniazid prophylaxis is recommended for HIV-infected patients with positive tuberculin skin tests.

**Jones DS (1992)**[62] studied 44 HIV infected children in Palm Beach country to estimate the prevalence of tuberculosis in them. They observed 36% of children were infected with tuberculosis and had pulmonary type of tuberculosis and also there was a increase in number of tuberculosis patients each year. They concluded that, largest effect

of the HIV epidemic on tuberculosis in children appeared to be indirect, through an increase in the number of adults with active tuberculosis serving as potential sources of tuberculosis infection for children.

**Chan SB (1996)**[24] studied the presentation of tuberculosis in 12 HIV infected pediatric patients in New York, the frequent presenting symptoms were fever (75%) and tachypnea (33%). Extrapulmonary TB was present in 3 cases (25%) and 4 cases (33%) of pulmonary tuberculosis. He concluded that children with AIDS and TB most frequently present with atypical manifestations of TB. A high index of suspicion is needed to correctly diagnose TB in this group of children. Early diagnosis is important because most respond well when treated appropriately.

**Lucas SB, Peacock CS, Hounnou A *et al*(1996)**[76] studied clinical presentations in 78 HIV infected pediatric patients in Abidjan. They observed pneumocystis carinii pneumonia, measles, pyogenic meningitis were the most common systemic lesions among the HIV positive patients and prevalence of tuberculosis, lymphocytic interstitial pneumonia and encephalitis were also seen in HIV positive patients.

**Nielsen K, Mcsherry G, Petru A *et al*(1997)**[92] screened 143 perinatally HIV infected pediatric patients in New Jersy. Recurrent bacterial infections, lymphoid interstitial pneumonia, wasting syndrome, mycobacterium avium infections, disseminated candida infections, pneumocystis carinii pneumonia, disseminated herpes simplex infections, disseminated cytomegalovirus infection, tuberculosis, cryptoccosis were the common clinical presentations seen in the HIV infected patients.

**Reddy BV, Ranganathan K, Kumarasamy N *et al*(1998)**[109] screened 13 HIV positive pediatric patients in Chennai. The common systemic lesion were pulmonary

tuberculosis, herpes zoster, lymphadenopathy, lower respiratory tract infections, staphylococcal skin infections.

**Spira R, Lepage P, Msellati P *et al*(1999)**[119] screened 218 HIV positive pediatric patients in Rwanda to assess the various systemic manifestations in HIV infected and uninfected pediatric patients. Oral candidiasis, chronic parotitis, hepatomegaly, generalized dermatitis, chronic fever, chronic diarrhea, lymphoid interstitial pneumonia, splenomegaly, lymphadenopathy, severe pneumonia, failure to thrive and chronic cough were the prevalent systemic presentations seen in their study.

**Dray SR, Lepage P, Dabis F (2000)**[31] studied the common systemic presentation in HIV pediatric patients in Africa. The common lesion observed were tuberculosis, which is the major cause for morbidity in the country. Common viral infections seen in them were measles, herpes zoster and herpes simplex infection. Pneumonia and influenza were the common bacterial infection observed in Africa and the common opportunistic infection was candidiasis. They conclude that the rate of morbidity and mortality in Africa can be reduced if the availability of antiretroviral drugs is increased and efficacy measures should be taken to reduce the perinatal transmission of HIV infection.

**Lodha R, Singhal T, Jain Y *et al*(2000)**[75] studied various clinical presentation seen in 27 HIV positive pediatric patients in New Delhi (India). The common clinical presentation seen in there study was failure to thrive in all patients, fever in 95.4% of patients, recurrent and persistent lower respiratory tract infection in 86.4% of cases, diarrhea in 45.5% of patients, lymphadenopathy was seen in 40% of cases. Other

systemic lesions observed were hepatomegaly, oral candidiasis, splenomegaly, disseminated TB and bronchiectasis.

**Agarwal M, Koppikar GV, Ghildiya Ri *et al*(2001)**[2] studied the clinical presentation in 821 HIV positive pediatric patients in Mumbai (India). Most of the patient in the study were between the age group of 1-3 years and the predominant clinical presentation observed in their study was chronic diarrhoea and disseminated tuberculosis. They concluded that the clinical presentation seen in HIV infected patients could be an additional pick up for detection of new HIV positive pediatric patients.

**Palme ID (2001)**[96] studied risk factors associated with seropositivity for HIV in Ethiopian children with clinical TB. HIV prevalence among children with clinical TB was 11.2%. High educational status of mothers, low age, loss of one or two parents and earlier Calmette-Guerin bacillus (BCG) vaccination of the child were factors independently related to HIV infection. They concluded that the higher education of parents, higher income and better living conditions may be the risk factors associated with TB seen in HIV infected pediatric patients.

**Merchant RH, Oswal JS, Bhagwat RV *et al(*2001)**[87] studied various systemic lesions in 285 HIV positive pediatric patients from various urban areas in Mumbai (India). Vertical mode of transmission was the most common mode acquiring HIV infection in their study. Most of the children infected were between the age group of 2-5years of age. The most common clinical presentation are protein energy malnutrition in 127 patients, pulmonary and extrapulmonary tuberculosis seen in 29.4% of cases, hepatomegaly in 28.7% of cases, generalized lymphadenopathy in 67 patients, skin lesion in 22.10% of cases. Other lesions observed were chronic diarrhea, oral thrush, unknown

pyrexia, chronic lung disease, chronic parotitis, otitis media, recurrent lower respiratory tract infection, pneumocystis carniii pneumonia were the common lesion observed in their study.

**Puthanakit T and Sirisanthana V (2003)**[105] in their retrospective study of 122 Thailand pedatirc HIV positive patients found that there was a decline in the number of cases reported with pneumonia but there is a marked increase in the incidence of TB in their country and this could be due to availability of antiviral drugs.

**Matid LH, Marcopito LF, Succi RC *et al*(2004)**[86] studied the clinical presentation in 914 HIV infected pediatric patients in Brazil. Multiple bacterial infection, pnemocystis carinii pneumonia, bacterial meningitis and interstitial lymphocytic pneumonia were the common presentations seen in their study.

**Swaminathan (2004)**[122] said that tuberculosis (TB) is the most common opportunistic infection in human immunodeficiency virus (HIV)-infected people worldwide. HIV-positive children are at risk of diagnostic error as well as delayed diagnosis of TB because of overlapping clinical and radiographic features with other lung diseases like Acute pneumonias and chronic lung diseases such as bronchiectasis and lymphocytic interstitial pneumonitis. TB manifestations are more severe in HIV-positive children and progression to death is more rapid than in HIV-negative children. He studied 62 children HIV positive children in Chennai, majority of them present clinically with pulmonary and extrapulmonary tuberculosis.

**Aghamohammadi A, Farhoudi A, Moin M *et al*(2005)**[1] screened 65 HIV positive Iranian pediatric patients. The most common systemic presentation in them was

otitis media, diarrhea, pneumonia, sinusitis, respiratory diseases and recurrent bacterial infections.

**Bavedekar SB and Agarwal R (2005)**[8] aimed to study the various systemic lesion in HIV infected patients and to find out the possible risk factor in them. They screened 115 HIV positive children in a hospital at Mumbai. Chronic fever, chronic diarrhea, servere malnutrition, persistent cough, oral thrush, generalized lymphadenopathy, generalized dermatitis, hepatomegaly, disseminated tuberculosis, were the common systemic infection observed in their study group. They found that oral thrush, generalized dermatitis and generalized lymphadenopathy were the significant independent clinical risk factors for predicting HIV seropositivity.

**Kumarasamy N, Vallabhaneni S, Flanigan TP *et al*(2005)**[73] 67 to 87 per cent of pediatric HIV infection is by Vertical transmission. Perinatally infected children become symptomatic by five years of age. Failure to thrive is the most common clinical condition associated with pulmonary and extra pulmonary tuberculosis. The most frequent opportunistic infection, oral candidiasis, hepatosplenomegaly, recurrent respiratory tract infection, Pneumocystis carinii pneumonia, chronic lung disease, persistent generalized lymphadenopathy, chronic diarrhoea, pyrexia of unknown origin, chronic hypertrophic parotitis, chronic ottorrhoea, bacterial skin infection, and PPE were noted .HIV epidemic grows and , it is important for primary care physicians to learn to suspect and test for HIV infection. Early detection of HIV optimizes chemoprophylaxis for opportunistic infections and provides an opportunity for secondary HIV prevention, with the availability of HAART; treatment can vastly reduce morbidity and mortality in Indian patients.

## CANDIDA IN HIV INFECTED PATIENTS

Oropharyngeal candidiasis may occur in up to 90% of human immunodeficiency virus (HIV)-infected patients during the course of the disease[32] Progressive cell-mediated immunodeficiency with decrease of CD4+ lymphocyte count to ≤ 200 cells/mm3 is a major risk factor for colonization with *Candida* species and development of candidiasis[74]. Clinically, OPC in HIV infection has been classified as exhibiting pseudomembranous and erythematous variants, or angular cheilitis

**Epstein JB, Pearsall NN, Truelove EL (1980)**[34] studied the CFU count in HIV positive adults and found that patients with candidiasis had >400 colony-forming units per ml of saliva, whereas carriers of candida albicans had <400 colony-forming units per ml. Thus, quantitative cultures of saliva may aid in the diagnosis of oral candidiasis.

**Challacombe SJ (1994)**[23] stated that candida infection in human is complex and both humoral immunity and cell mediated immunity place a major role. Humoral immunity is protective by salivary antibodies and serum antibodies, which prevents adhesion of the fungi. Cell mediated immunity plays a role particularly in chronic hyperplastic candidiasis.

**Lynch DP and Teen M (1994)**[78] Candida albicans is ubiquitous dimorphous yeast and has the potential to cause human diseases under specific circumstances and conditions. The usual presentation is pseudomembraneous candidiasis and erythematous candidiasis.

**Soll DR, Morrow B, Srikantha T (1994)**[118] stated that candida albicans and related species switch frequently and reversibly between a number of general phenotypes usually discriminated by colony morphology. They isolated two genes PEP1 and Op4,

which are responsible for this morphological shift and these genes are isolated from the HIV infected patients.

**Cannon RD, Holmes AR, Mason AB *et al* (1995)**[22] Candida albicans is frequently isolated from the human mouth, yet few carriers develop clinical signs of candidiasis. Oral candidiasis presents clinically in many forms. This reflects the ability of the yeast to colonize different oral surfaces and the variety of factors, which predispose the host to candida colonization and subsequent infection. Colonization of the oral cavity appears to be facilitated by several specific adherence interactions between C. albicans and oral surfaces, which enable the yeast to resist host clearance mechanisms.

**Monteil R and Madinier I *et al*(1997)**[88] observed that C.albicans and C.krusei are the common candidal species isolated from HIV negative patients.

**Sweet SP (1997)**[123] stated that candida is a harmless commensal of oral cavity. They are virulent in patients whose immune status is low as in case of HIV infection. Pathogenecity depends on the hyphal formation, thigmotrophism, protease secretion, adherence and phenotypic switching.

**Masur H (1999)**[85] the most common oppurtiunistic infection in HIV infected patients is oral candidiasis, which can be used to assses the immune status of the patients. It is usually treated using cotrimazole and it shows various degrees of resistance.

**Bartie KL, Williams DW, Wilson MJ *et al* (2000)**[6] They studied the ability of candida albicans isolates to invade an in vitro oral tissue model and they correlated it with the infection origin particularly to hyperplastic candidiasis (CHC). Reconstituted human oral epithelium was infected with C. albicans isolated from normal oral mucosa, CHC, non-CHC oral candidiasis and squamous cell carcinoma (SCC; n ¼ 4). Differential

patterns of invasion were evident and, whilst consistent for a given isolate, did not relate to the infection origin of the isolate. Two principal patterns of invasion were evident and described as either a 'localised' or a 'uniform' distribution of invading hyphae. Several isolates also exhibited superficial infection with limited hyphal invasion. In conclusion, the use of the in vitro tissue model allowed the assessment of the invasive capabilities of isolates of C. albicans. However, the apparent differences in invasive characteristics did not appear to be related to the clinical origin of isolates.

**Krautgarner WD, Hanning M, Weitgasser R *et al* (2001)**[72] stated that candida attaches to the epithelium or to any keratinized structure in the body by two ways either with fimbria or gets attached superficially. Fimbria mediated adhesion enables colonization of the epithelial surface and by invasion of the superficial epithelial cells via hyphae.

**Maartens G (2002)**[79] said that various opportunistic infections associated with HIV infections in sub-Saharan Africa differ markedly in their incidence from those in industrialized countries. Tuberculosis, microsporidiosis, pneumocystis carinii are the commonest cause of morbidity and mortality.

## CANDIDA IN HIV PEDIATRIC PATIENTS AND TREATMENT:

Fungal infections in HIV infected individuals are associated with advancement of disease. In pediatric HIV infections, symptomatic children have a significantly highly incidence of clinical candidiasis and persistent drug resistant candidiasis than do asymptomatic HIV infected children[103]. Candida is a harmless commensal of oral cavity they are virulent in patients whose immune status is low as in case of HIV infection. Pathogenicity depends on the hyphal formation, thigmotrpism, protease secretion, adherence and phenotypic switching[78]. When the sign and symptoms are atypical, clinical diagnosis is verified by microscopic examination and culture[118]. The yeast identification depends on the physician; the timely identification of the organisms by a laboratory can aid in making the correct diagnosis[69]. The usual species isolated from HIV pediatric patients are Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis and Candida krusei[93].

**Viscoli C, Castagnola E, Fioredda F *et al*(1991)**[133] stated that fluconazole, a new triazole derivative, was evaluated in a pilot study of 34 episodes of candidiasis in 24 children. Clinical and microbiological success was achieved in 30 of 34 cases. Fluconazole might represent an effective alternative to amphotericin B in the treatment of candidiasis in children. Comparative trials are necessary to assess optimal dosages and efficacy.

**Deborah G (1994)**[50] Oral candidiasis is a pathognomonic sign of immunocompromised patients. It occurs in two forms in oral cavity as the pseudomembranous and erythematous form. Preferred treatment for oral candidiasis is cotrmoxiazole, candida isolated from HIV positive patients develop resistance soon.

**Kolokotronis A, Kioses V, Antoniades D, Mandraveli K (1994)**[69] They relate the relationship between oral candidiasis and hairy leukoplakia, circulating CD4 cells and serum anti p24 antibodies in 43 HIV positive patients. They found that these two lesions occur when the immune status of the patient is low and absence of antip24 antibodies influenced by low $CD4^3$.

**Pfaller M A, Houston A, Coffmann S (1996)**[100] CHROMagar Candida is a new differential culture medium that allows selective isolation of yeasts and simultaneously identifies colonies of *Candida albicans*, *C. tropicalis*, and *C. krusei*. They evaluated the use of this medium with 316 yeast isolates including 247 isolated directly on CHROMagar from clinical material. A total of 43 of the 234 positive cultures contained mixtures of yeast species. Twenty (47%) of these mixed cultures were detected only on CHROMagar. CHROMagar is extremely useful in making a rapid presumptive identification of common yeast species. This capability plus the ability to detect mixed cultures of *Candida* spp. promises to improve and streamline the work flow in the mycology and clinical microbiology laboratory.

**Hick MJ, Carter AB, Rossman SN *et al*(1998)**[54] determined the prevalence of fungal organisms in 27 HIV infected pediatric patients and correlated it with the severity of immunosuppression state. They found that the amount of fungal organisms isolated from severely immunosuppressed patients were more than the other two groups.

**Velegraiki A, Nicolatous O, heodoridou M *et al* (1999)**[129] screened 4 HIV positive pediatric patients who had linear gingival erythema. They could to isolate C.dubliniensis from 3 patients. They concluded that C.dubliniensis could be the cause for

linear gingival erythema seen in these patients and could be a variant of erythematous candidiasis.

**Pelletier R, Peter J, Antin C *et al*(2000)**[98] Oropharyngeal candidiasis (OPC) is a common opportunistic infection in human immunodeficiency virus (HIV)-infected patients and other immunocompromised hosts. Cotrimazole troches are widely used in the treatment of mucosal candidiasis. The authors investigated the potential emergence of resistance to cotrimazole in a prospectively monitored HIV-infected pediatric population receiving this azole. They compared MICs in macrodilution and microdilution assays. They further analyzed the correlation between these in vitro findings and the clinical response to antifungal therapy in 87 HIV-infected children. They conclude that resistance to cotrimazole develops in isolates of *C. albicans* from HIV-infected children, that cross-resistance to other azoles may develop concomitantly, and that this resistance correlates with refractory mucosal candidiasis.

**Reichart Pa, Samarnayaka LP, Philipsen HP *et al*(2000)**[111] in there studies stated that since erythematous candidiasis posses only blastospores they are difficult to grow in laboratory conditions.

**Nailum ER and Cittukr FG (2001)**[90] oral candidiasis is an early sign of illness or disease progression in HIV/AIDS and other immunocompromised states. Emergence of new candida species, drug resistance and immature immune systems add to the complexity of this condition, especially in children. Candida species accounts for 40% of normal oral flora. Peseudomembranous candidasis, erythematous candidiasis and hyperplastic candidiasis are the common candidal carriage seen in HIV infected patients. Linear gingival erythema is considered to be one of the type of candidiasis.

**Campisi G, Pizzo G, Milici ME *et al*(2002)**[20] assessed the candidal carriage rate in HIV infected patients and correlated with the CD4 cell count. Carriage rate was 61.9% and 29.3% in HIV positive individual and there was a significant correlation between the candidal carriage rate and the CD4 count.

**Priscilla DL, Milan EP, Martinez R *et al*(2002)**[104] the authors studied the prevelance of candida in 130 positive patients, used chromagar to identify them and found that candida albicans was the common species to be isolated.

**Starr JR and White TC (2002)**[120] Little is known about carriage of *Candida albicans*, the predominant pathogenic yeast in oral infection, in children. They cultured buccal mucosal and gingival swabs from 150 normal Portuguese children to investigate the prevalence of *C. albicans* at baseline (before dental treatment), post-treatment, and 12, 24, and 36months post-baseline. The children, aged 8 to 11years at baseline, had no systemic disease or clinical symptoms of oral candidiasis and there was decrease in the amount of candida before and after treatment.

**Vargas KG and Joly S (2002)**[128] Candida samples were taken from 54 HIV positive patients and studied the yeast carriage, intensity of carriage and genotypes overtime. They found the intensity of carriage was significantly increased in symptomatic positive patients than in the negative patients. They isolated candida albicans and noted that there was a change in the species of candida with elapse of time. They concluded that commensal strain colonizing in HIV positive patient can undergo alterations prior to producing an episode of thrush.

**Bosa VL, Birman EG, Cury AE *et al*(2003)**[15] studied oral fungal microbiota in 30 HIV infected pediatric patients and compared them with the HIV negative patients using sugar assimilation test, germ tube test, carbon and nitrogen assimilation test. C.albicans and C.parpsilosis, C.tropicalis were the common species isolated from the HIV positive patients and C.albicans, C.guilliermondii were the common species isolated from the HIV negative pediatric group.

**Kim JO, Garofalo L, Shelly DB *et al*(2003)**[66] isolated Candidia dubliniensis from 14 HIV positive patients out of 205 samples using API system. The candida dubliniensis is a recently described specie that share many phenotypic and morphological features with Candida albicans.

**Reichart PA (2003)**[110] stated that oral candidiasis (OC) is a frequent oral manifestation of HIV infection and is a marker for disease and occurs as pseudomembranous, erythematous or rarely hyperplastic variant; angular cheilitis is also seen. Candida albicans is frequently isolated but other species such as C. krusei and C. dublienensis are emerging. Linear gingival erythema, necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis have been described in HIV-infected patients. AIDS-associated Kaposi's sarcoma (KS) predominantly occurs on the palate, the gingiva and the dorsum of the tongue. Histopathologically, oral KS is identical to classical KS. Oral KS has been treated surgically, using laser, radiotherapy and intralesional injections with chemo- and immunotherapy. After introduction of highly active antiretroviral therapy (HAART) oral manifestations, such as OC, gingivo-periodontitis and KS are rarely seen.

**Portela MB, Souza IPR, Costa EB *et al* (2004)**[103] they isolated candida species from 52 HIV positive pediatric patients from the subgingival area using CHROMagar. Candia albicans was the common species of Candida isolated. Candida glabrata, Candida tropicalis, Candida dubliniensis were the other common species isolated.

**Salobrena AC, Cepeda LG, Laura C et *al* (2004)**[116] investigated the temporal changes in the prevalence of oral candidiasis and they found that oral candidiasis was prevalent in this study group. No significant variations in incidence were noted after HAART theapy. They found that candidiasis was the common fungal disease in HIV patients and they do not decrease significantly after HAART.

**Villar CC, Kashleva H, Bagtzoglous DA *et al*(2004)**[132] candida albicans with the true hypha can attach with the normal or injured epithelium. By stimulating the release of cytokines they cause injury to the epithelium. They conclude saying that candida with true hypha is responsible for causing clinical lesion.

**Hung C, Yang Y, Lauderdale TL *et al* (2005)**[58] explained the *Candidal* colonization in HIV infected outpatients in Taiwan. The authors conducted a prospective cohort study of *Candida* colonization and its risk factors. 50% of the patients were colonized with *Candida* species. They found that antibiotic treatment and lower CD4 counts (<200 cells/mm3) increased the rate of oropharyngeal candidiasis in HIV-infected patients, while antiretroviral therapy protected patients from the development of candidiasis.

## TECHNIQUES FOR ISOLATION AND IDENTIFICATION OF CANDIDA SPECIES:

There are a variety of methods available for identifying yeast from clinical specimens. The germ tube morphology studies and carbohydrate utilization and commercially available methods eg. CHROMagar, API 20, Uni-yeast Tek are used to identify the yeast morphology[9]. Enzymatic and fluorogenic test are conventional methods. These systems are commercial systems, which give quick results[64]. Each method has its advantages and limitations and frequently more than one method may be required to identify an organism to the species level.

**Katsura Y and Uesaka I (1974)**[64] estimated the severity of clinical candidal lesion by estimating the free dispersed germ tube in the serum. They found that more the density of germ tube in the serum more will be the severity of the clinical lesion. This test is useful in estimating the seropositivity of deep seated candidiasis.

**Fleming WH, Hopkins JM, Land GA (1977)**[40] used new medium named as corn meal agar that provides rapid presumptive identification of Candida albicans C. albicans is differentiated from other yeasts by the sequential production of germ tubes and chlamydospores. In a comparison with cornmeal agar control plates, there was an increase of chlamydospore-forming strains of C. albicans and a decrease in the time required for chlamydospore formation. This system helps to identify different candidal species.

**Berardinelli S and Opheim DJ (1985)**[11] used a new germ tube induction medium, composed of three parts Rabbit Coagulase Plasma with EDTA and two parts Tryp-Soy broth and this was effective for the presumptive identification of Candida

albicas. This medium was safer to use, more accurate, and less expensive than other commercial germ tube induction media. This medium helps to identify Candida albicans.

**Fung JC, Donta ST, Tilton RC (1986)**[42] Eighty-three serum specimens from 24 patients infected with Candida albicans were with the Candida Detection System (CAND-TEC). The medical records of each patient were reviewed for clinical evidence of Candida colonization or disease. CAND-TEC showed high titer in patients with disseminated candidiasis. This technique appears to be a useful, rapid, and noninvasive means of laboratory diagnosis of systemic candidiasis.

**Perry JL, Miller GR, Carr DL (1990)**[99] isolated a total of 706 yeast strains and isolated were evaluated in parallel by the Candida albicans screen test and germ tube in comparison with the API20C yeast identification system. Germ tube was correctly identified in 419 samples. Germ tube negative was seen in Candida tropicalis and Candida parapsilosis. Sensitivity and specificity of both the systems is 98%.

**Odds FC and Benaerts R (1994)**[93] used CHROMagar candida which is a novel, differential culture medium that is claimed to facilitate the isolation and presumptive identifications of candidal species. Out of 726 samples, which were incubated for 48hrs in 37degree centigrade. C.tropicalis produced blue colonies on the agar plate, C.krusei produced pink colonies, C.albicans produced green colonies. The major disadvantage of this medium is that it can identify only few variants of candidal species.

**Beighton D, Ludford R, Clark DT et *al* (1995)**[10] used a new differential medium, CHROMagar Candida, for the isolation of clinically important yeasts. They wanted to determine its usefulness in facilitating the study of oral yeasts. They recovery of yeasts on the medium was not significantly different from the recovery on Sabouraud

dextrose agar. *Candida* (*Torulopsis*) *glabrata*, *Candida parapsilosis*, *Candida magnoliae*, *Candida lusitaniae*, *Candida famata*, *Candida kefir*, and *Saccharomyces cerevisiae* were readily distinguished from *C. albicans* and *C. tropicalis* isolates. One of the major disadvantage is that number of species identified is very less and it is very difficult to differenciate Candida albicans from Candida dubliniensis. But still it is a very useful medium for the study of yeasts associated with dental diseases.

**Kirkpatrick WR, Revankar SG, Mcatee RK *et al* (1995)**[67] stated that Candida dubliniensis has phenotypic characters similar to that of Candida albicans. The authors screen 63 HIV positive patients for Candida dubliniensis and they used chrom agar. They could isolate Candida dubliniensis in 3 patients.

**Crist AE, Johnson LM and Burke PJ (1996)**[28] The Microbial Identification System was evaluated for the identification of 550 clinically isolated yeasts. The organisms evaluated were fresh clinical isolates identified by methods routinely used in our laboratory (API 20C and conventional methods) and included *Candida albicans* (*n* 5 294), *C. glabrata* (*n* 5 145), *C. tropicalis* (*n* 5 58), *C. parapsilosis* (*n* 5 33), and other yeasts (*n* 5 20). Of the 550 isolates tested, 374 (68.0%) were correctly identified to the species level, with 87 (15.8%) being incorrectly identified and 89 (16.2%) giving no identification. The most frequently misidentified yeast was *C. glabrata*, which was identified as *S. cerevisiae* 32.4% of the time. On the basis of these results, the MIS, with its current database, does not appear suitable for the routine identification of clinically important yeasts.

**Heelan JS, Siliezar D, and Coon K (1996)**[52] observed that the germ tube production is a method for the presumptive identification of *Candida albicans* has been in use for many years. Methods have recently been developed for detecting the production of the enzymes L-proline aminopeptidase and b-galactosaminidase by yeast isolates grown in culture. Both enzymes are produced by *C. albicans*; other yeasts may produce either L-proline aminopeptidase or b-galactosaminidase but not both enzymes. One hundred thirty-three clinical yeast isolates, including 55 *C. albicans*, 27 *Candida tropicalis*, 22 *Torulopsis* (*Candida*) *glabrata*, and 29 other yeast isolates were tested by the germ tube production method and three tests for enzyme production, with the API 20C method used as a "gold standard." All three enzymatic methods evaluated provided more objective and rapid nonmicroscopic alternatives to the germ tube test and may be used to accurately distinguish *C. albicans* from other yeasts

**Pfaller M A, Houston A, Coffmann S (1996)**[100] used CHROMagar Candida a new differential culture medium that allows selective isolation of yeasts and simultaneously identifies colonies of *Candida albicans*, *C. tropicalis*, and *C. krusei*. They evaluated the use of this medium with 316 yeast isolates including 247 isolated directly on CHROMagar from clinical material. Over 95% of stock and clinical isolates of *C. albicans*, *C. tropicalis*, and *C. krusei* were correctly identified on the basis of colony morphology and pigmentation on CHROMagar. Additionally, CHROMagar also allowed the identification of *C.* (*Torulopsis*) *glabrata* at a similar level of accuracy. This capability plus the ability to detect mixed cultures of *Candida* species promises to improve and streamline the work flow in the mycology and clinical microbiology laboratory.

**Campbell CK, Davey KG, Holmen AD *et al*(1999)**[19] compared the efficiency of commercial yeast identification system (API system) with the conventional system for 145 candidal samples. They found that false positive results were more in commercial system than the conventional system.

**Gales C, Pfaller A, Houston AK, Joly S *et al*(1999)**[43] Tried to Identifiy Candida *dubliniensis* based on Temperature and Utilization of Xylose and a-Methyl-D-Glucoside as determined with the API 20C AUX and Vitek YBC Systems. To have a better understanding of the role of *Candida dubliniensis* in clinical infections, it is essential that microbiology laboratories can identify this species rapidly and accurately in clinical specimens. *C. dubliniensis* has been reported to lack the ability to utilize xylose (XYL) and a-methyl-D-glucoside (MDG) and to grow poorly or not at all at 45°C, whereas *Candida albicans* isolates utilize XYL and MDG and usually grow well at 45°C. they tested 66 isolates of *C. dubliniensis* and 100 isolates of *C. albicans* with both the API 20C AUX and Vitek YBC systems to evaluate the ability of the XYL and MDG tests contained within each of these systems to distinguish between the two species. Clinical microbiology laboratories could use lack of growth at 45°C and a negative XYL test with either the API 20C AUX or Vitek yeast identification system to provide a presumptive identification of *C. dubliniensis*. A negative MDG test result with either system would also be helpful but may misclassify *C. albicans* as *C. dubliniensis*, especially when the API 20C AUX system is used.

**Koehler AP, Chu K, Elizabeth T, Houang S and Cheng AB (1999)**[68] cornmeal identified the candidal species by using the morphological features were as CHROMagar identified them with the help of different colours for different species. For the

identification of Candida species corn meal agar, CHROMagar and API system can be used.

**Pincus D H, Coleman D C, Pruitt W R *et al*(1999)**[101] These authors tested the sensitivity between conventional techniques and the commercial available products for isolation of C.dubliniensis in 210 candidal samples. They found that the sensitivity of commercial system (API 20C AUX, ID32 C, RapID Yeast Plus, VITEK YBC, and VITEK 2 ID-YST systems) is more and rapid than the conventional system and the differentiation between the C.albicans and C.dubliniensis is good in commercial system than the conventional system.

**Wadlin JK, Hanko G, Stewart R *et al*(1999)**[134] Two-hundred and one yeast isolates were compared with three commercial systems RapID Yeast Plus System with 3 commercial system namely: Innovative Diagnostic Systems, Norcross, Ga.; API 20C Aux; bioMerieux-Vitek, Hazelwood, Mo.; and Vitek Yeast Biochemical Card, bio-Merieux-Vitek against an auxinographic and microscopic morphologic reference method for the ability to identify yeasts commonly isolated in clinical microbiology laboratory. The RapID Yeast Plus System was significantly better than either API 20C Aux (193 versus 167 correct identifications) or the Vitek Yeast Biochemical Card (193 versus 173 correct identifications; *P* 5 0.003) for obtaining correct identifications to the species level without additional testing. There was no significant difference between results obtained with API 20C Aux and the Vitek Yeast Biochemical Card system. The API 20C Aux system did not correctly identify any of the *Candida krusei* isolates (*n* 5 23) without supplemental testing and accounted for the major differences between the API 20C Aux

and RapID Yeast Plus systems. Overall, the RapID Yeast Plus System was easy to use and is a good system for the routine identification of clinically relevant yeasts.

**Brown DM, Rizk MJ, Falkler Wa *et al*(2000)**[18] They used immune system and suppressed cellular immunity in children HIV infection to provide disease progression. They isolated candida in 47 patients using rinse technique. CHROMagar, ATCC 49256, sugar assimilation test were used to identify the Candida species. They found that Candida glabrata, is the common species identified. Candida dubliniensis were isolated in 3 patients.

**Hidalgo HF, Orenga S, Lebeau B *et al*(2001)**[56] used Candida ID system, a new chromogenic medium for the identification of *Candida albicans* (blue colonies) and preliminary identification into a group of four species (pink colonies). They compared it with Albicans ID2 and Sabouraud gentamycin chloramphenicol. Out of 446 fungal strains, Candida ID allowed the isolation of more species than Albicans ID 2 (95.5% versus 91.2%).

**Kim JO, Garofalo L, Blecker DS *et al*(2003)**[66] They studied the candida dubliniensis retrospectively from the samples that are previously identified as Candidia abicans. They studied about 183 samples and isolated this species in 5 of the patients but there was no significant clinical relevance. They concluded that further investigation might help to find the relevance of these species in pediatric group.

# *Materials & Methods*

***STUDY DESIGN:*** Prospective study, 150 consecutive HIV seropositive patients over a period of one year were enrolled by using convenience sampling technique.

***STUDY SUBJECTS:*** HIV seropositive pediatric patients from Government TB sanatorium, Tambaram, Government Institute of Children Health, Egmore, YRG Care VHS, Taramani and Ragas Dental Collage and Hospital, Uthandi, constituted the study group.150 patients between 6 months to 15 years of age who were confirmed HIV seropositive either by ELISA / Western blot / Tridot, tests were included. Informed verbal consent was taken from the patient / guardian for clinical examination, photography and for collecting salivary samples. All the clinical details were noted in a preformatted case sheet. Systemic lesions were diagnosed based on the clinical diagnosis given by the pediatrician of the respective hospital. All oral lesions were diagnosed based on the diagnostic criteria formulated by EC Clearing House (Annexure III)

***SAMPLE COLLECTION***: Salivary samples for candida evaluation were collected from the oral cavity from both symptomatic and asymptomatic HIV seropositive pediatric patients using swab technique (Samaranayake swab technique)

**PROCEDURE:** *Step 1:* Sterile disposable cotton swab was used to collect samples. In symptomatic patients the sample was collected from the lesional area. In asymptomatic patients the sample was collected from the dorsum of the tongue.

*Step 2*: The collected sample was inoculated in the Sabourauds Dextrose Agar (SDA) and incubated at 37 $^{0}$C for 48 hours.

*Step 3:* The morphology of the colonies and the Colony Forming Unit (CFU) are determined.

*Step 4:* *G*erm-tube test was performed to identify the pseudohypha forms, which indicates that the yeast formed is candida albicans.

*Step 5:* The colonies are selected randomly from the Sabouraud Dextrose Agar plate and inoculated in the CHROMagar and incubated at 37 $^{0}$C for 48 hours. The color of the colonies was noted.

*Step 6:* Species that could not be identified by CHROMagar were subjected to cornmeal test. In this test, the samples were incubated at 37 $^{0}$C for 48 hours. The morphology of the species was studied under light microscope.

**MATERIALS:**

***SAMPLE COLLECTION:***

1. Sterilized disposable cotton swab.

***GERM TUBE TEST AND PREPARATION OF REAGENTS:***

2. Disposable Petri dish 15ml size
3. Conical flask 300ml
4. Distilled water 5liters
5. Chloramphenicol capsules
6. Bunsen burner
7. Autoclave
8. Physical balance
9. Disposable 15ml vials
10. Human serum 10 ml
11. Microscopic glass slide
12. Cover slip
13. Light microscope
14. Petri dish 15ml
15. Bacterial loop
16. Conical flask 300ml

***REAGENTS:***

1. Sabourauds Dextrose Agar (SDA 15 grams)
2. CHROMagar Candida
3. Cornmeal Agar

***METHODS:***

***Specimen Collection:***

All the oral candidial samples were collected from seropositive patients using disposable cotton swabs. ***(Figure 24)***

***SDA agar composition and preparation:***

| | | |
|---|---|---|
| Mycological peptone | _ | 10gm / L |
| Dextrose | _ | 40gm / L |
| Agar | _ | 15gm / L |

- 65 gm of SDA agar was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. The medium was sterilized by autoclaving at 15 Ibs pressure, 121 $^{0}$C for 15 minutes, then poured into sterile Petri dishes.
- After 48 hrs of incubation of sample streak on the medium at 37$^{0}$C, the positive cultures showed individual colonies that were cream / pale brown, smooth / glabrous, moist / membranous appearance. ***(Figure 25 & 26)***
- Quantification of colonies was done by calculating the number of colony forming units per ml (CFUs).
- These colonies were smeared on the slides, stained (Gram stain) and examined microscopically, they showed characteristic round to ovoid yeast forms of candida.

**Germ-tube Test:**

- Representative colony were taken in an inoculation loop and dissolved in disposable plastic vials containing 2ml of human serum.
- This colony were incubated for 2 to 4 hours at $37^0$C
- The vial was shaked well, using disposable micropipettes 10microliters / ml of the solution was taken, one wet smear and one smear stained by Grams stain was made. ***(Figure 27 & 28)***
- The smear was studied under microscope for presence of pseudohyphae and true hyphae

**Interpretation:**

Pseudohyphae: filamentous, cylindrical outgrowth from the yeast cells with no constriction present at the base. It helps to differentiate C.albicans from the other species of candida.

***CHROMagar Candida Composition and Preparation:***

| | | |
|---|---|---|
| Agar | _ | 15 gm / L |
| Peptone | _ | 10.2 gm / L |
| Chromogenic Mix | _ | 22gm / L |

- CHROMagar Candida powder was dissolved in distilled water in the proportion of 47.7 gm/ L and heated to boiling to dissolve the medium completely. Large bubbles replace foam in about 2 minutes, and it should not be heated to more than $100^0$C. The media was cooled in water bath and poured into sterile Petri dishes.
- After 48 hours of incubation at $37^0$C, presumptive identification of yeast isolates was done based on the color of the colonies. ***(Figure 29 & 30)***

**Interpretation:**

- Green _ C. albicans
- Blue _ C. tropicalis
- Pink _ C. krusei
- White to pink _ Other species.

***Corn meal agar test:***

**Composition**:

Cornmeal : 8gm
Agar : 4gm
Distilled water : 200ml
Tween 80[1%] : 2gm

The above mentioned ingredients were mixed in a conical flask and autoclaved at 121°C for 15 minutes and poured into petri plates. Candida species were identified according to the microscopic morphological features on the cornmeal Tween80 agar.

- An isolate from SDA was taken and inoculated by making 3 parallel cuts about half an inch apart at 45° into the cornmeal culture plate.
- The plate was incubated at 30°C for 48 hours. After 48 hours, heavy inoculum of yeast was streaked across the plate .A glass slide and cover slip were placed over it and examined under 10X at the edge of the cover slip. ***(Figure 31)***
- Candida species were identified based on the microscopic morphological features.

**Interpretation:**

**Candida albicans:** Chlamydospores present, abundant pseudohyphae, some true hyphae, clusters of blastospores. ***(Figure 33)***

**Candida krusei:** Extensive branched pseudomycelium with chain of elongated cells giving tree like appearances, clusters and chains of blastospores along with pseudohyphae.

**Candida tropicalis:** No pseudohyphae, often radiating with clusters of blastoconida at center. ***(Figure 34)***

**Candida parapilosis:** Branched chains of elongated cells with clusters of blastospores along them, occasional giant cells. ***(Figure 32)***

# *Statistical Analysis*

- Data entry, database management and analysis was done using SPSS version 10.0.5.
- Chi-square test was utilized to find out the statistical significant difference between gender in age, religion, birth status, route of transmission, HIV status of father, mother and siblings, pattern of feeding, neonatal jaundice, past medication, present medication, CD4 count, systemic lesions, oral lesions, clinical candidal type, candidal status and candidal species.
- Clinical candidal type by age, candidal species by site and candidal species by gender difference also found by chi-square test.
- Student t-test was applied to find out the mean CFU count difference between candidal status, gender and hemoglobin.

# *Results*

150 pediatric patients were enrolled in this study. Of the 150 patients, 82 (55.7%) were males and 68 (45.3%) were females. 87 (58.0%) were between the age group of 6-12 years [45 (54.9%) males and 42 (61.8 %) females]. 2 (1.3 %) patients were less then 12 months of age. ***(Table 1 Graph 1)***

Birth status of 148 patients was recorded. 9 (6.0%) had history of premature birth; 139 (92.7%) had full term birth status. Birth status of 2 (1.3%) patients was not available as they were orphans. ***(Table 1 Graph 2)***

143 (95.3%) patients had acquired HIV infection via vertical transmission. 6 (4.0%) had a history of blood transfusion. For one (0.7%) patient who was an orphan the route of transmission was not available ***(Table 1, Graph 3)***

HIV status of the parents were available for 150 patients. 117 (78%) children, the mother and the father were HIV positive. 6 (4.0%) children acquired HIV infection via blood transfusion and HIV status was not ascertained for the parents of 2 patients. The HIV status of the parents of 150 children were as follows: 118 (78.7%) fathers and 148 (98.7%) mothers were positive. ***(Table 1, Graph 4)***

Of the 55 patients siblings for whom HIV status was available, 15 (27.3%) siblings tested negative for HIV infection and 12 (21.8 %) tested positive. ***(Table I, Graph 5)***

Feeding pattern the children was recorded in 128 (85.3%) patients. 81 (54%) children were breast fed, 12 (8%) were only bottle fed and 35 (23.3%) were both breast and bottle fed. ***(Table 1)***

Of the150 children, neonatal jaundice was noted in 32 (39%) males and 29 (42.6%) females. ***(Table 1)***

The past medical history revealed that 78 (52%) patients received ATT drugs and 11 (7.3%) were given alternative medications (Siddha & Homeopathy medicine). One (0.7%) patient had received ART treatment for 6months. ***(Table 2, Graph 6)***

At the time of examination, 126 (84%) were taking antibiotics, 123(82%) were taking multivitamins and 76 patients (50.7%) were on Iron supplements. 74 (72.5%) patients were on ATT, 68 patients (45.3%) were taking Antifungal therapy, 13(8.7%) patients were taking Acyclovir and 61 (40.7%) patients were receiving ART drugs. ***(Table 3, Graph 7)***

Immune status of 150 patients showed that 37 (39.4%) were moderately immune suppressed, 34 (36.2%) had severe immune suppression and 23 (24.5%) had no immune suppression. ***(Table 4, Graph 8)***

Systemic lesions were observed in 150 pediatric patients. Lymphadenopathy ***(Figure1)*** was the most common systemic lesion seen in 125 (83.3%) [69 (84.1%) males and 56 (82.4%) females]. Tuberculosis (TB) was the next most prevalent lesion seen in 91 patients (60.7%). Of which pulmonary tuberculosis (PTB) was the most common presentation. Of the 32 patients (21.3%) with PTB, there were 23 males (28.1%) and 9 females (13.2%) (p=0.02). Out of 68 (45.4%) patients with cold abscess ***(Figure 2 & Figure 3)***, 67 patients (44.7%) had cold abscess of the cervical group of lymph nodes [40 (48.8%) were males and 27 (39.7%) were females] and only one female patient had cold abscess involving the axillary group of lymph node. Primary complex was seen in 38 (25.3%) patients [males 18 (22%), females 20 (29.4%)], 73 patients (48.7%) had otitis media [males 40(48.8%), females 33(48.5%)], mumps in 37 patients (24.7%) [males 17 (20.7%), females 20 (29.4%)], parotitis ***(Figure 4)*** was seen in 28(18.7%) patients

[ males 17(20.7%), females 11(16.2%)] impetigo ***(Figure 5)*** was seen in 4(2.7%) patients and scabies ***(Figure 6)*** was seen in 23 (15.3%)[males 14(17.1%), females 9(13.2%)]. Puritic eruptions ***(Figure 12)*** was seen in 77 (51.3%) of patients [male 46(56.1%) and females 31(45.6%), Oropharyngeal Candidiasis ***(Figure 7)*** was documented in only 4 (5.9%) female patients (p= 0.04). Vaginal Candidiasis was seen in 4 (5.9%) female patients. Herpetic eruption ***(Figure 8),*** Herpes Zoster was observed in 6 (4%) of patients [males 2(2.4%) and females 4 (5.9%)] **(*Figure 9 & 10)***, warts and bronchitis had equal prevalence in both the genders [Male 1 (1.5%), Female 1 (1.5%)]. There was one case of molluscum contagiosum ***(Figure 11)*** 1 (1.2%) and it was in a male patient. ***(Table 5, Graph 9)***

Oral lesions were noted in all the 150 pediatric patients. Oral candidiasis was the most common lesion seen in 97 patients (64.7%) [males 54 (65.4%), females 43 (63.2%)]. The most prevalent type of candidiasis was pseudomembranous candidiasis (PC) ***(Figure 13 & 14)*** and it was seen in 85 (56.7%) [males 44 (53.7%), females 41(60.3%)]. Angular cheilitis (AC) ***(Figure 17)*** was the next common candidial type seen in 36 patients (24%) [males 25(30.5%), females 11 (16.2%)] (p=0.054). erythematous candidiasis (EC) ***(Figure 15)*** was seen in 33 (22%) [males 16(19.5%), female 17(25%)], PC and EC was seen in 11(7.3%)[males 7 (8.5%), females 4 (5.9%)] and 2(1.3%) patients had hyperplastic candidiasis (HC) ***(Figure 16)*** [male 1(1.2%), female 1(1.5%)]. Conventional gingivitis was the next common lesion noted in 14 patients (9.3%) [males 6(7.3%), females 8 (11.8%)]. There were 12 (8.0%) cases of pigmentation ***(Figure 18 & 19)*** [males 9(11%), females 3(4.4%)], 9(6%) cases of depapillation of the dorsum of the tongue ***(Figure 21 & 22)*** [males 8(8.5%), females 2 (2.9%)]. OHL ***(Figure 20)*** was seen

in 8 patients (5.3%) [males 5(6.1%), females 3(4.4%)] and ulcers were seen in 8 patients (5.3%) [males 4(4.9%), females 4 (4.9%)]. Kaposi's sarcoma was seen in one male patient. ***(Table 6, Graph 10)***

The distribution of clinical candidal type observed in 87 (58%) patients between the age group of 6-12 are PC in 46 (52.9%) patients, EC in 20 patients (23%), HC in 1 patient (2.1%), AC in 19 (21.8%) patient and a combination of PC and EC in 10 (11.5%) patients. The clinical candidal type observed in 61 (40.7%) patients between the age group of 1-5years were, PC 37(60.7%) patients, EC 12(19.7%) patients, HC 1(1.6%) patients, AC in 16 (26.2%) of patients and PC and EC was seen in 1 (1.6%). Only 2 (1.6%) patients in our study were less than 12 months of age and the clinical variant of candidal type observed in this age group was AC in one patient (50%) and PC and EC in one patient (50%). ***(Table 7 &Graph 11)***

Out of 150 patients, candida samples were collected from 122 patients who fulfilled the inclusion criteria. Of the 122, 66(54%) were males and 56 (45.9%) were females.

Out of 122 patients for whom the candida samples were obtained, 36 (29.5%) were clinically asymptomatic for candida infections and 86 (70.5%) were clinically symptomatic. ***(Table8, Graph 12)***

The candidal species isolated from 122 patients were C.albicans, C.tropicalis and C.krusei ***(Figure 29)***. C.albicans was isolated in 32 patients (26.2%) [males 17 (25.8%), females 15 (26.8%)]. C.tropicalis was isolated in 24 (19.7%) [males 11 (16.7%), females 13 (23.2%)] C.krusei was identified in 5 (4.1%) patients[4 (6.1%) were male and 1 (1.8%) was a female]. Mixed colonies of C.albicans with C.krusei ***(Figure 30)*** were isolated from

2 patients (1.6%) [males 1(1.5%), females 1 (1.8%). C.albicans with C.tropicalis were isolated in 8 patients (6.6%) [males 3(4.5%), females 5(8.9%)]. Mixed colonies of non-specific species with C.albicans was isolated from 4(3.3%) patients [males 2(3%), females 2(3.6%)] and along with C.tropicalis was seen in 2(1.6%) and both were male patients. Mixed colonies of C.krusei with C.tropicalis (Figure 30) was isolated from 2 patients (1.6%) [male 1(1.5%), female 1(1.8%)] Non-specific species ***(Figure 30)*** were isolated in 8 patients (6.6%) [male 5(7.6%), females 3(5.4%)]. ***(Table 9, Graph 13)*** . Cornmeal test was done for the 8 nonspecifice species isolated from CHROMagar.

Of the 36 (29.5%) asymptomatic patients, Candida samples for all of them were taken from dorsum of the tongue. The species isolated were C.albicans 6 (16.7%), C.tropicalis 6(16.7%) and C.krusei 2 (5.6%). Mixed colonies of C.tropicalis with a non specific species was isolated from one (2.8%) patient. ***(Table 10 & 11, Graph14& 15)*** The mean Colony Forming Unit (CFU) in this group was $6.269 \pm 3.06$.

The clinical presentation of candidal infection in 86 (70.5%) symptomatic patients were as follows, 54 patients had PC on the dorsum of the tongue. The candidal species isolated were C.albicans in 20 patients (37%), C.tropicalis in 11 patients (20.4%), C.krusei in 3 patients (5.6%) and non specific species in 7(13%) patients. 2 patients who had PC on the lateral surface of the tongue, the species isolated was C.albicans 2(100%). Out of 30 patients who had PC on the dorsum of the tongue and EC on the hard palate, 4 patients had EC only on the hard palate. The candidal species isolated in these samples was C.albicans in one patient and C.tropicalis in other patients (50%). 26 patients who had both PC and EC the species isolated were C.albicans in 3(11.5%), C.tropicalis in 6 patients (23.1%), mixed colonies of C.albicans with C.krusei in 2 patients (7.7%) and

with C.tropicalis in 8 patients (30.8%), nonspecific species with C.albicans in 4 patients (15.4%) and with C.tropicalis in 1(3.8%) were isolated. 2(7.7%) patients had mixed colonies of C.krusei with C.tropicalis (p=0.00). ***(Table 10 & 11, Graph 14& 15)*** The mean CFU in the symptomatic group was 16.06± 3.17.

There was a statistical significant difference in the mean CFU count between the symptomatic and asymptomatic patients (p=0.00). ***(Table 12, Graph 16)***

The mean CFU count of candida for the 122 patients was 14.70 ± 5.05 in females and 12.09 ± 5.30 in males (p= 0.076). **(*Table 13, Graph 17)***

The mean CFU for clinical candidal type were, HC (17.80 ± 2.26), PC (16.31 ± 3.09), PC and EC was (16.05 ± 3.34). EC had the least mean CFU count of (14.62 ± 3.12). ***(Table 14, Graph 18)***

The patients with severe immunosuppression had a mean CFU count of 4.81 x $10^3$ cfu/ml. In patients with no suppression the CFU count was 6.35 x $10^3$ cfu/ml and in moderately suppressed immune patients the CFU count was 4.21 x $10^3$ cfu/ml. ***(Table 15, Graphs19)***

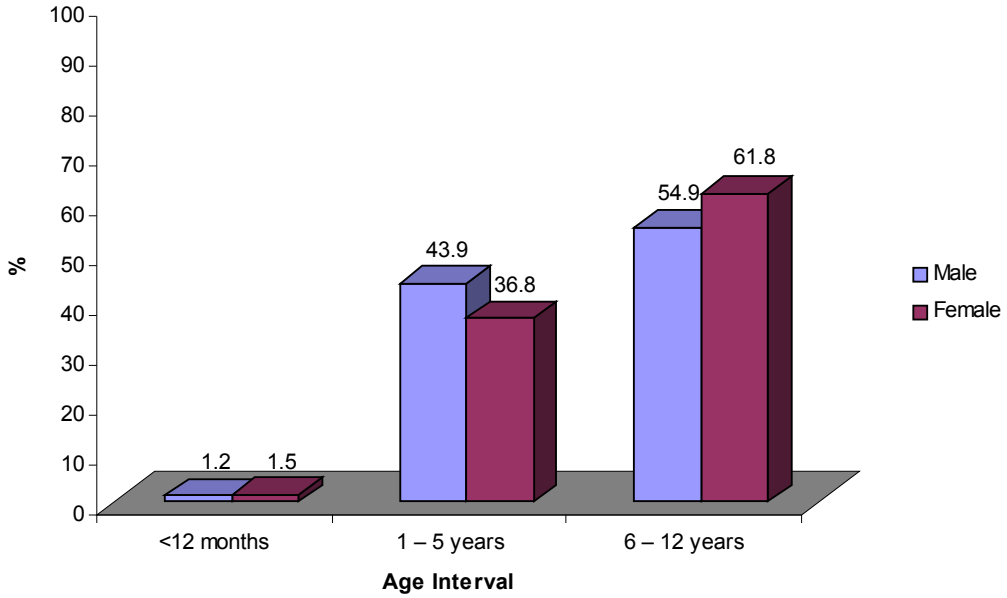
In our study, patients with normal hemoglobin level had CFU count of 13.61 ± 5.62 and in patient who are clinically anaemic the CFU count was 15.1 ± 3.57. ***(Table 16, Graph 20)***

*Tables & Graphs*

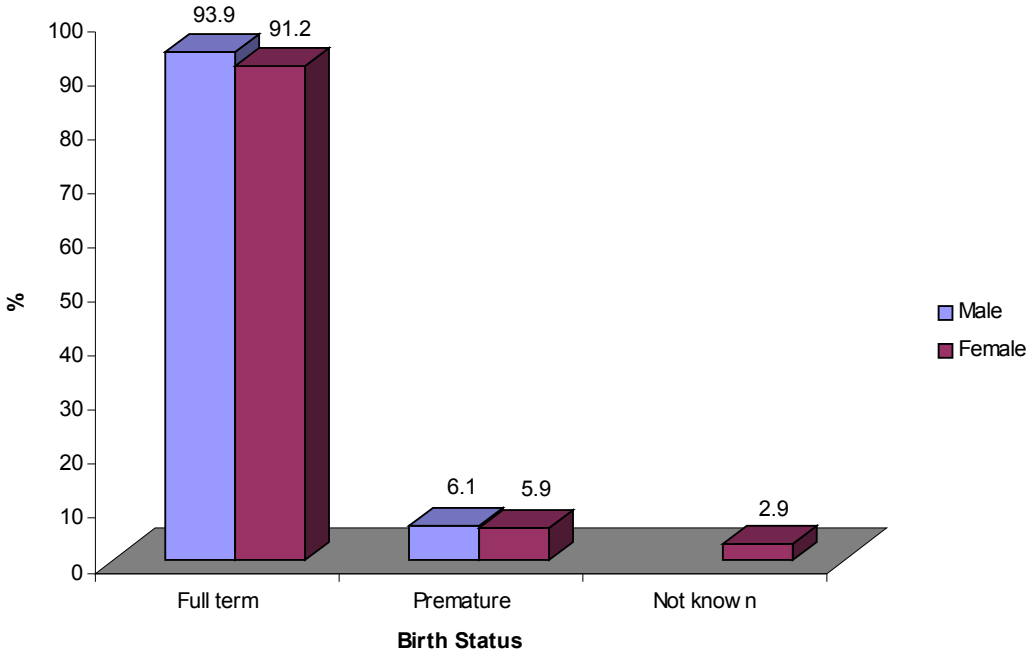
**Table 1: HIV infected pediatric patients - Demographic characteristics (n= 150)**

| Variables | | Male n=82 | (%) 45.7% | Female n=68 | (%) 45.3% | p value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|---|
| ***Age*** | <12 months | 1 | 1.2 | 1 | 1.5 | 0.675 | 2 | 1.3 |
| | 1 – 5 years | 36 | 43.9 | 25 | 36.8 | | 61 | 40.7 |
| | 6 – 12 years | 45 | 54.9 | 42 | 61.8 | | 87 | 58.0 |
| ***Religion*** | Hindu | 76 | 92.7 | 55 | 80.9 | 0.083 | 131 | 87.3 |
| | Christian | 5 | 6.1 | 12 | 17.6 | | 17 | 11.3 |
| | Muslim | 1 | 1.2 | 1 | 1.5 | | 2 | 1.3 |
| ***Birth status*** | Full term | 77 | 93.9 | 62 | 91.2 | 0.295 | 139 | 92.7 |
| | Premature | 5 | 6.1 | 4 | 5.9 | | 9 | 6.0 |
| | Not known | - | - | 2 | 2.9 | | 2 | 1.3 |
| ***Route of transmission*** | | | | | | 0.459 | | |
| | Vertical | 78 | 95.1 | 65 | 95.6 | | 143 | 95.3 |
| | Blood | 4 | 4.9 | 2 | 2.9 | | 6 | 4.0 |
| | Unknown | - | - | 1 | 1.5 | | 1 | 0.7 |
| ***HIV status of Father*** | | | | | | 0.269 | | |
| | Positive | 61 | 87.1 | 56 | 94.9 | | 118 | 78.7 |
| | Negative | 5 | 7.1 | 1 | 1.7 | | 6 | 4.7 |
| | Not tested | 4 | 5.7 | 2 | 3.4 | | 6 | 4.7 |
| | Total | 70 | 100 | 59 | 100 | | 129 | 100 |
| ***HIV status of Mothers*** | | | | | | 0.703 | | |
| | Positive | 81 | 98.8 | 67 | 98.5 | | 148 | 98.7 |
| | Negative | 1 | 1.2 | 1 | 1.5 | | 2 | 1.3 |
| ***HIV status of Siblings*** | | | | | | 0.696 | | |
| | Positive | 5 | 17.2 | 7 | 26.9 | | 12 | 21.8 |
| | Negative | 9 | 31.0 | 6 | 23.1 | | 15 | 27.3 |
| | Not tested | 4 | 13.8 | 2 | 7.7 | | 6 | 10.9 |
| ***Pattern of Feeding*** | | | | | | 0.988 | | |
| | Bottle | 6 | 7.3 | 6 | 8.8 | | 12 | 8.0 |
| | Breast | 45 | 54.9 | 36 | 52.9 | | 81 | 54.0 |
| | Combination | 19 | 23.2 | 16 | 23.5 | | 35 | 23.3 |
| **Neonatal jaundice** | | 32 | 39.0 | 29 | 42.6 | 0.739 | 61 | 40.7 |

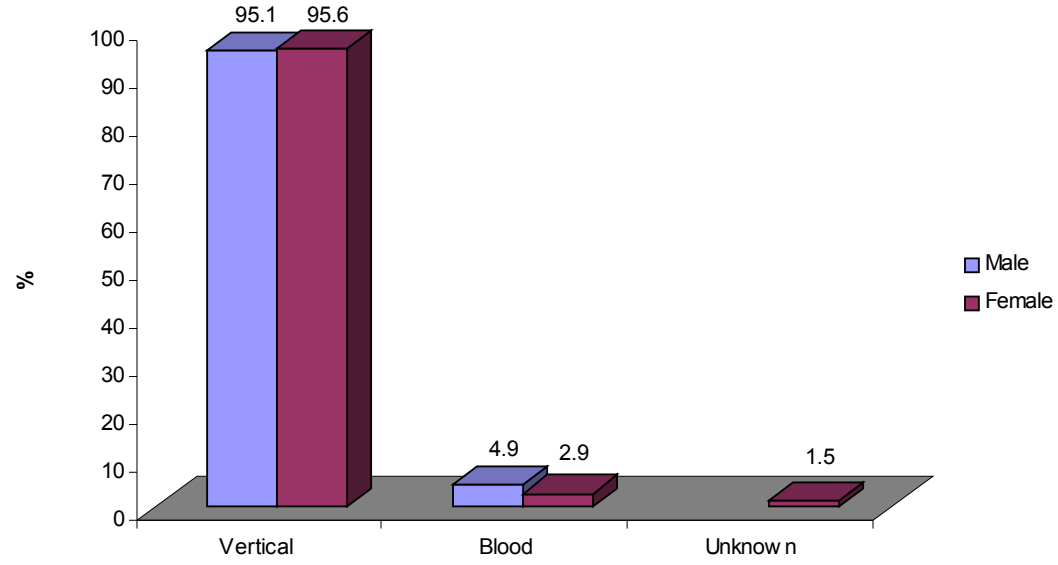
**Graph 1: HIV infected pediatric patients - Age and gender distribution**



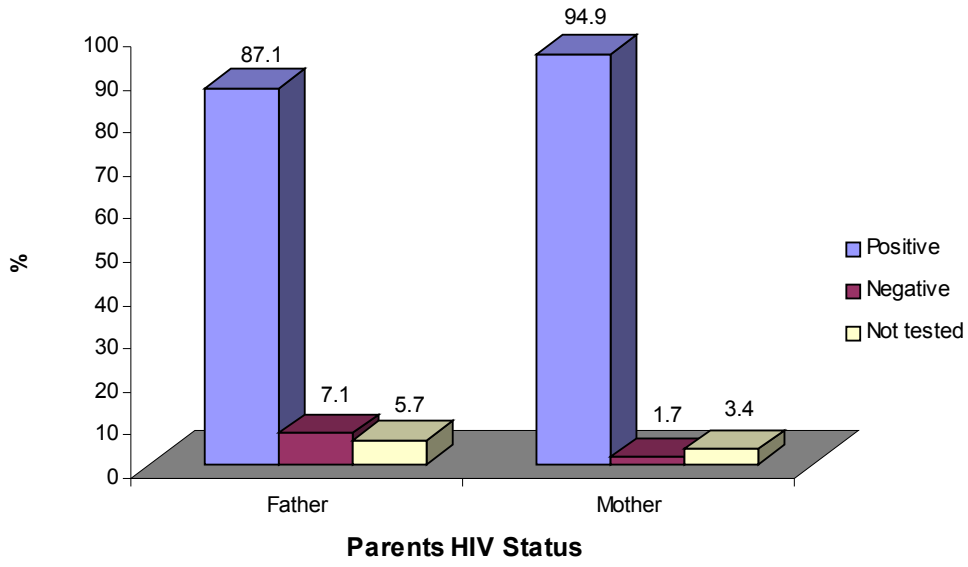

**Graph 2: HIV infected pediatric patients - Birth Status**



**Graph 3: HIV infected pediatric patients - Route of Transmission**




**Graph 4: HIV infected pediatric patients - HIV status of Parents**



**Graph 5: HIV infected pediatric patients - HIV status of the siblings**

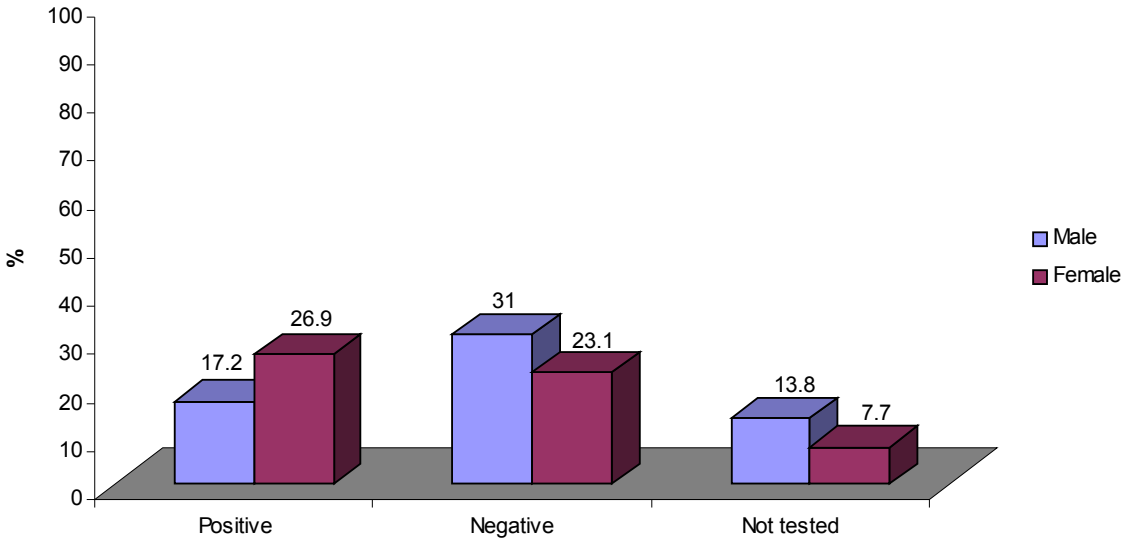


**Table 2: HIV infected pediatric patients - Past medication (n=150)**

| Past medication | Male n=82 | (%) | Female n=68 | (%) | p value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|
| Anti tuberculous treatment | 40 | 48.8 | 38 | 55.9 | 0.229 | 78 | 52.0 |
| ART | - | - | 1 | 1.5 | | 1 | 0.7 |
| **Others:** Antibiotics | 1 | 1.2 | - | - | | 1 | 0.7 |
| Not known | 32 | 39.0 | 27 | 39.7 | | 59 | 39.3 |
| **Alternative medications** | 4 | 4.9 | 2 | 2.9 | | 6 | 4.0 |
| Homeopathy | 5 | 6.1 | - | - | | 5 | 3.3 |
| Siddha | | | | | | | |

**** Since 6month before the date of examination**

**Graph 6: HIV infected pediatric patients - Past medication**

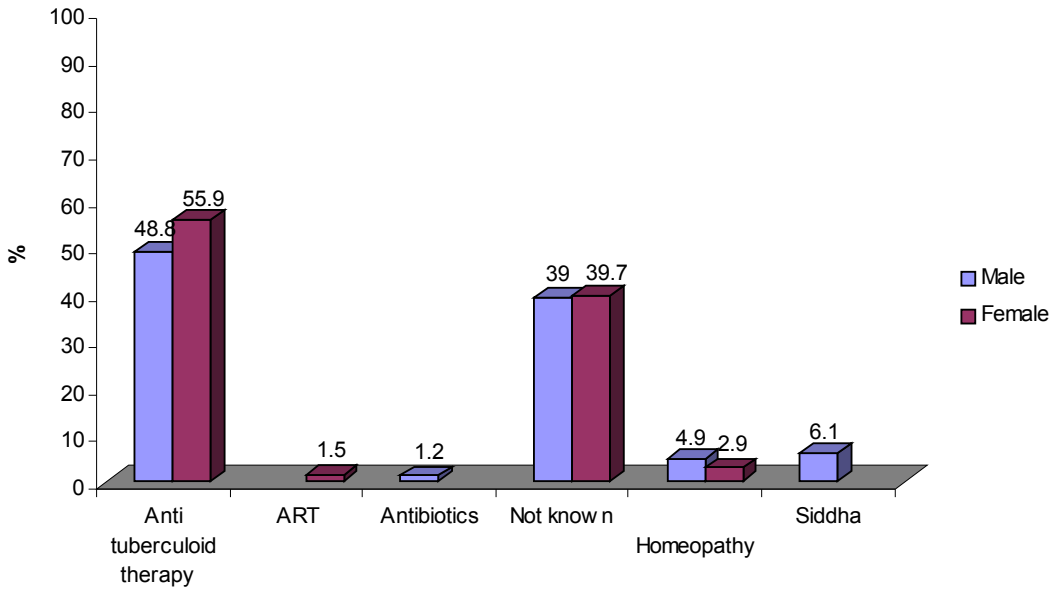


**Table 3: HIV infected pediatric patients - Present medication (n=150)**

| Present medication | Male n=82 | (%) | Female n=68 | (%) | P value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|
| Antibiotics | 69 | 84.5 | 57 | 83.8 | 0.565 | 126 | 84.0 |
| Multivitamin | 64 | 78.0 | 59 | 86.8 | 0.203 | 123 | 82.0 |
| Antifungal dugs | 36 | 43.9 | 32 | 47.1 | 0.412 | 68 | 45.3 |
| Iron supplement | 41 | 50.0 | 35 | 51.5 | 0.494 | 76 | 50.7 |
| Anti tuberculous treatment | 38 | 69.1 | 36 | 76.6 | 0.505 | 74 | 72.5 |
| Anti viral drugs (Acyclovir) | 5 | 6.1 | 8 | 11.8 | 0.174 | 13 | 8.7 |
| ART | 32 | 39.0 | 29 | 42.6 | 0.388 | 61 | 40.7 |

**[**]At the time of examination**

**Graph 7: HIV infected pediatric patients - Present medication**

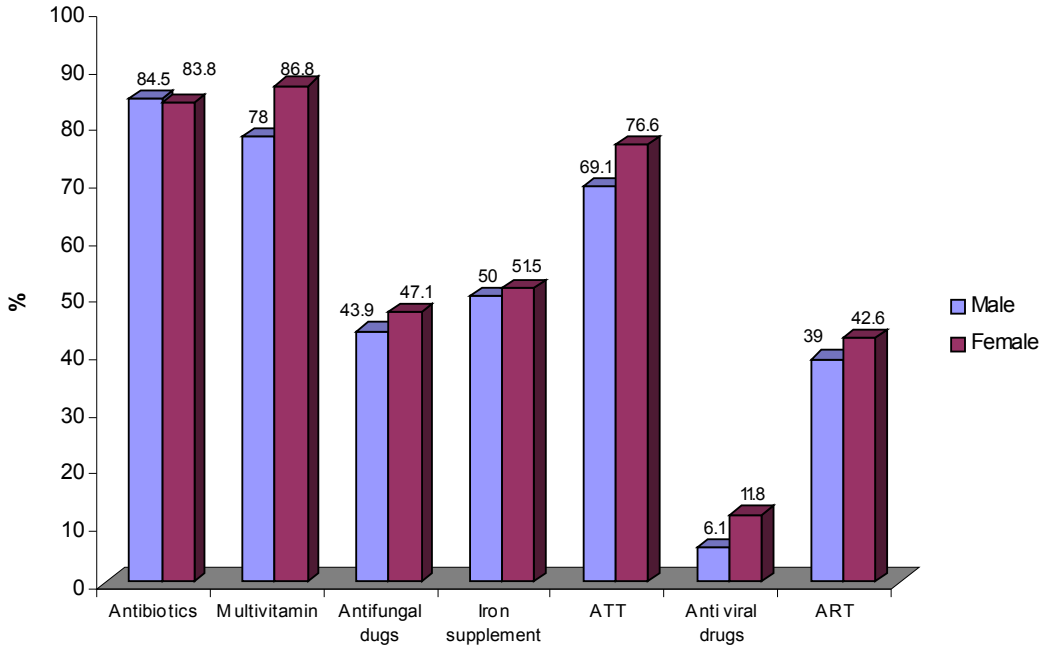


**Table 4: HIV infected pediatric patients - Immune status (n=150)**

| CD4 COUNT | | Male n=82 | (%) | Female n=68 | (%) | P value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|---|
| *CD4* | No suppression | 14 | 27.5 | 9 | 20.9 | 0.634 | 23 | 24.5 |
| | Moderate | 18 | 35.3 | 19 | 44.2 | | 37 | 39.4 |
| | Sever | 19 | 37.3 | 15 | 34.9 | | 34 | 36.2 |

**Graph 8: HIV infected pediatric patients - Immune status**

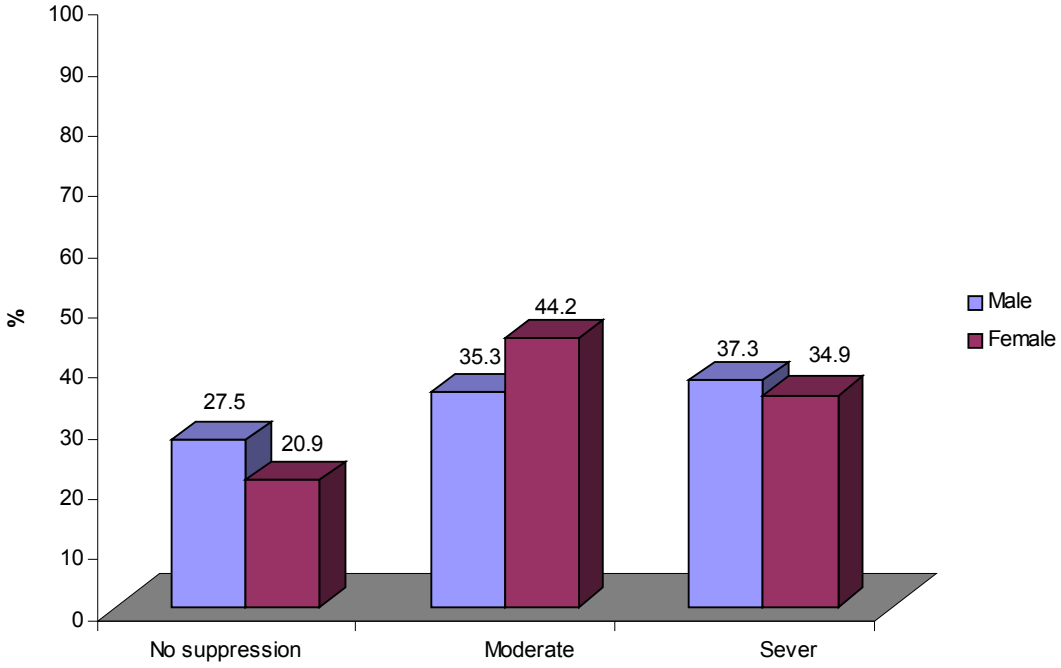


**Table 5: HIV infected pediatric patients - Prevalence of systemic lesions (n=150)**

| Systemic lesions | Male n=82 | (%) | Female n=68 | (%) | p value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|
| Lymphadenopathy | 69 | 84.1 | 56 | 82.4 | 0.828 | 125 | 83.3 |
| **Tuberculosis (TB)** | 50 | 61.0 | 41 | 60.3 | 1.000 | 91 | 60.7 |
| Pulmonary TB | 23 | 28.1 | 9 | 13.2 | 0.02* | 32 | 21.3 |
| Primary complex | 18 | 22.0 | 20 | 29.4 | 0.347 | 38 | 25.3 |
| Cold abscess | 40 | 48.8 | 27 | 39.7 | 0.323 | 67 | 44.7 |
| *Cervical TB* | 1 | 1.2 | - | - | 1.000 | 1 | 0.7 |
| *Axillary TB* | | | | | | | |
| Measles | 49 | 59.8 | 39 | 57.4 | 0.868 | 88 | 58.7 |
| Otitis media | 40 | 48.8 | 33 | 48.5 | 1.000 | 73 | 48.7 |
| Puritic eruption | 46 | 56.1 | 31 | 45.6 | 0.132 | 77 | 51.3 |
| Upper respiratory tract infection | 32 | 39.0 | 22 | 32.4 | 0.495 | 54 | 36.0 |
| Mumps | 17 | 20.7 | 20 | 29.4 | 0.256 | 37 | 24.7 |
| Parotitis | 17 | 20.7 | 11 | 16.2 | 0.532 | 28 | 18.7 |
| Scabies | 14 | 17.1 | 9 | 13.2 | 0.650 | 23 | 15.3 |
| Pneumonia | 10 | 12.2 | 7 | 10.3 | 0.799 | 17 | 11.3 |
| Herpes Zoster | 2 | 2.4 | 4 | 5.9 | 0.257 | 6 | 4 |
| Vaginal candidiasis | - | - | 4 | 5.9 | 0.04* | 4 | 2.7 |
| Impetigo | 4 | 4.9 | - | - | 0.127 | 4 | 2.7 |
| Oral pharyngeal candidiasis | - | - | 4 | 5.9 | 0.04* | 4 | 2.7 |
| Systemic candidiasis | 1 | 1.2 | 1 | 1.5 | 1.000 | 2 | 1.3 |
| Bronchitis | 1 | 1.2 | 1 | 1.5 | 1.000 | 2 | 1.3 |
| Herpetic eruption | 1 | 1.2 | 1 | 1.5 | 1.000 | 2 | 1.3 |
| Common wart | 1 | 1.2 | 1 | 1.5 | 1.000 | 2 | 1.3 |
| Molluscum Contagiosum | 1 | 1.2 | - | - | 1.000 | 1 | 0.7 |
| Any systemic lesion | 82 | 100 | 68 | 100 | 1.000 | 150 | 100 |

*** p value < 0.05 Statistically significant**

**Graph 9: HIV infected pediatric patients - Prevalence of systemic lesions**

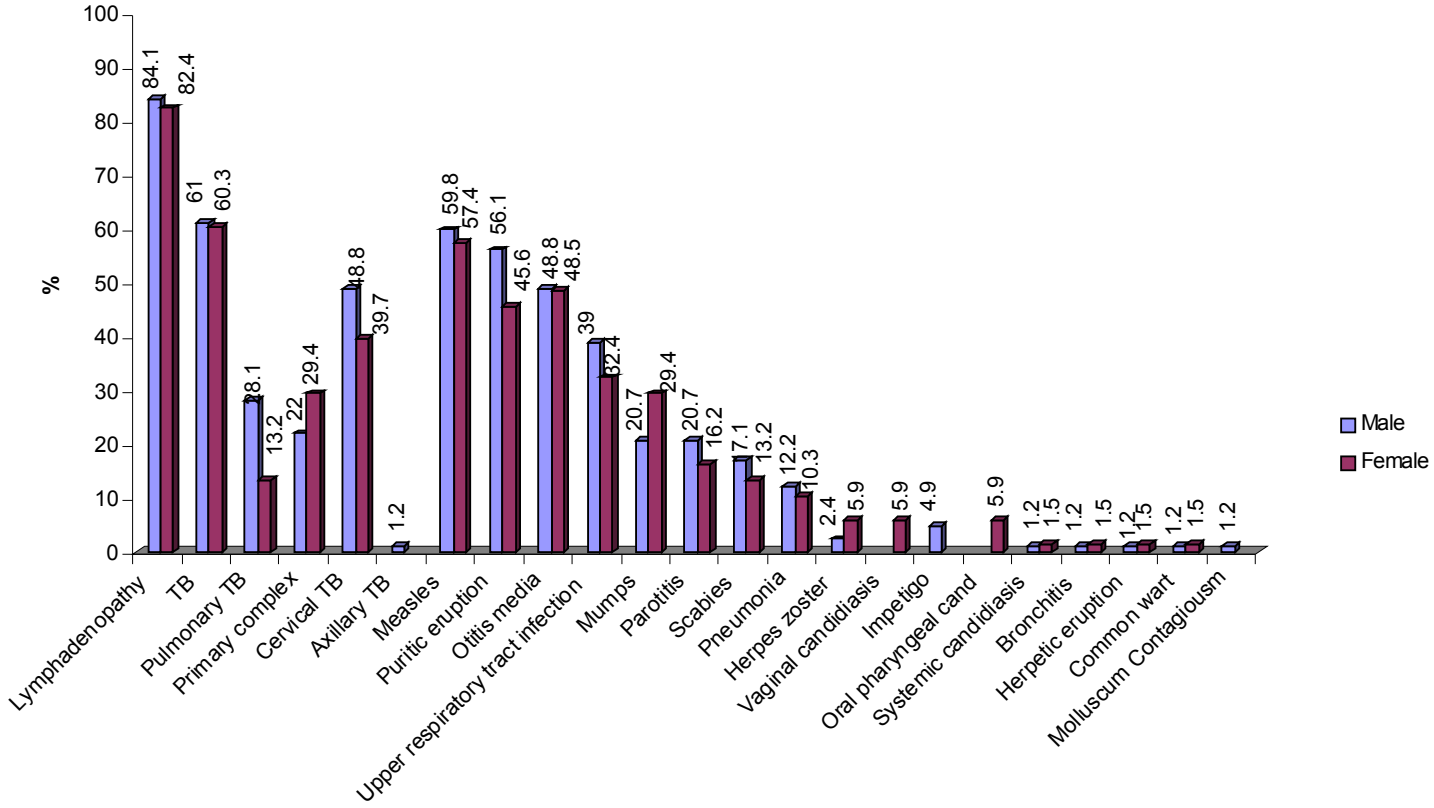


**Table 6: HIV infected pediatric patients - Prevalence of oral lesions (n=150)**

| Oral lesions | Male n=82 | (%) | Female n=68 | (%) | p value | Total n=150 | (%) |
|---|---|---|---|---|---|---|---|
| Oral candidiasis | 54 | 65.4 | 43 | 63.2 | 0.864 | 97 | 64.7 |
| *PC** | 44 | 53.7 | 41 | 60.3 | 0.508 | 85 | 56.7 |
| *EC** | 16 | 19.5 | 17 | 25.0 | 0.436 | 33 | 22.0 |
| *PC + EC** | 7 | 8.5 | 4 | 5.9 | 0.755 | 11 | 7.3 |
| *HC** | 1 | 1.2 | 1 | 1.5 | 1.000 | 2 | 1.3 |
| *AC** | 25 | 30.5 | 11 | 16.2 | 0.054 | 36 | 24.0 |
| Pigmentation | 9 | 11 | 3 | 4.4 | 0.226 | 12 | 8.0 |
| Others ( Depapillation ) | 8 | 8.5 | 2 | 2.9 | 0.11 | 9 | 6.0 |
| Gingivitis | 6 | 7.3 | 8 | 11.8 | 0.406 | 14 | 9.3 |
| Oral hairy leukoplakia | 5 | 6.1 | 3 | 4.4 | 0.73 | 8 | 5.3 |
| Ulcer | 4 | 4.9 | 4 | 4.9 | 1.000 | 8 | 5.3 |
| Kaposi's sarcoma | 1 | 1.2 | - | - | 1.0 | 2 | 1.3 |
| Any oral lesion | 82 | 100 | 68 | 100 | 1.000 | 150 | 100 |

***PC –** Pseudomembraneous candidiasis

**EC –** Erythematous candidiasis

**HC –** Hyperplastic candidiasis

**AC –** Angular chelitis

**PC + EC –** Pseudomembraneous candidiaisis & Erythematous candidiasis

**Graph 11: HIV infected pediatric patients - Prevalence of oral lesions**

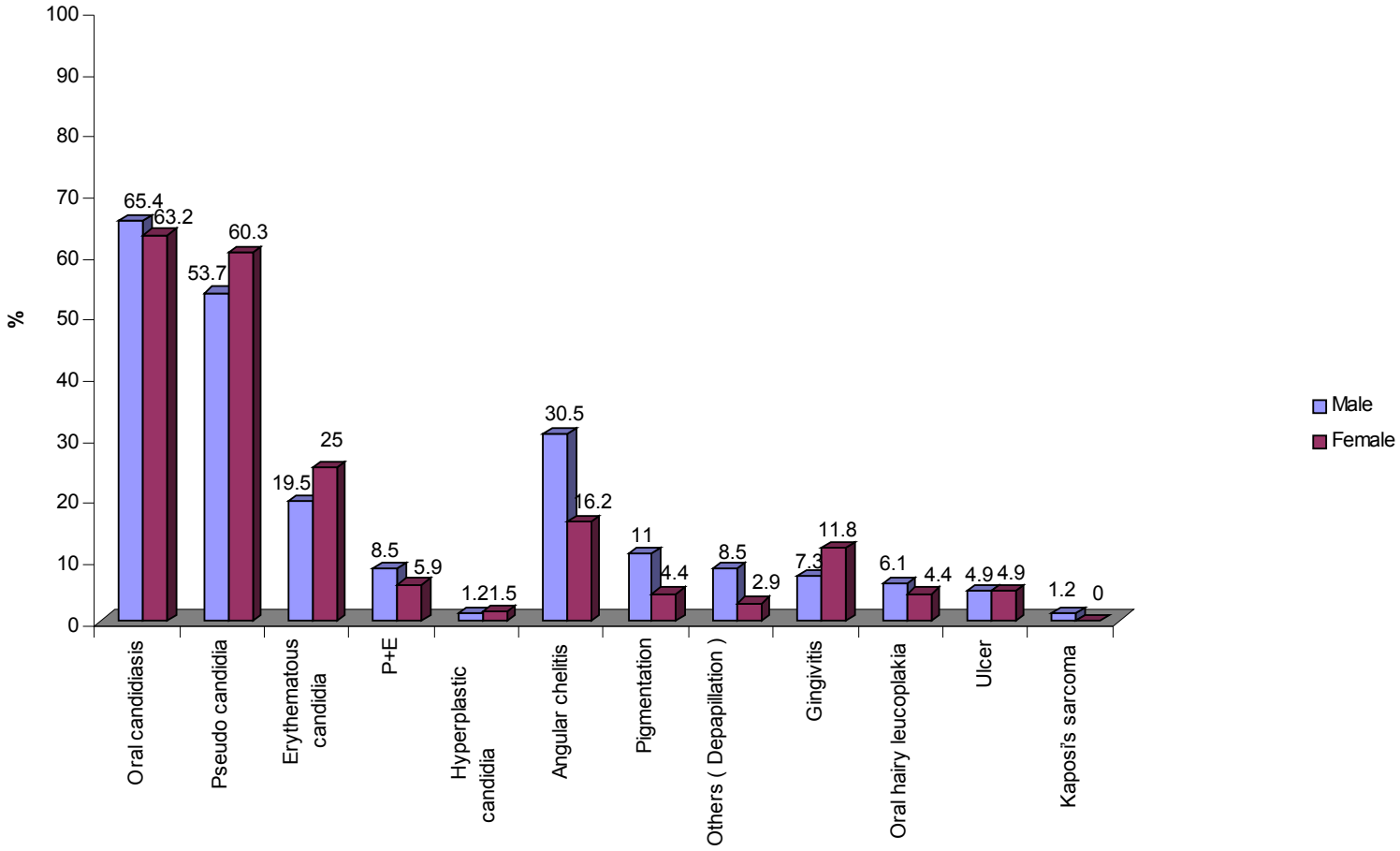
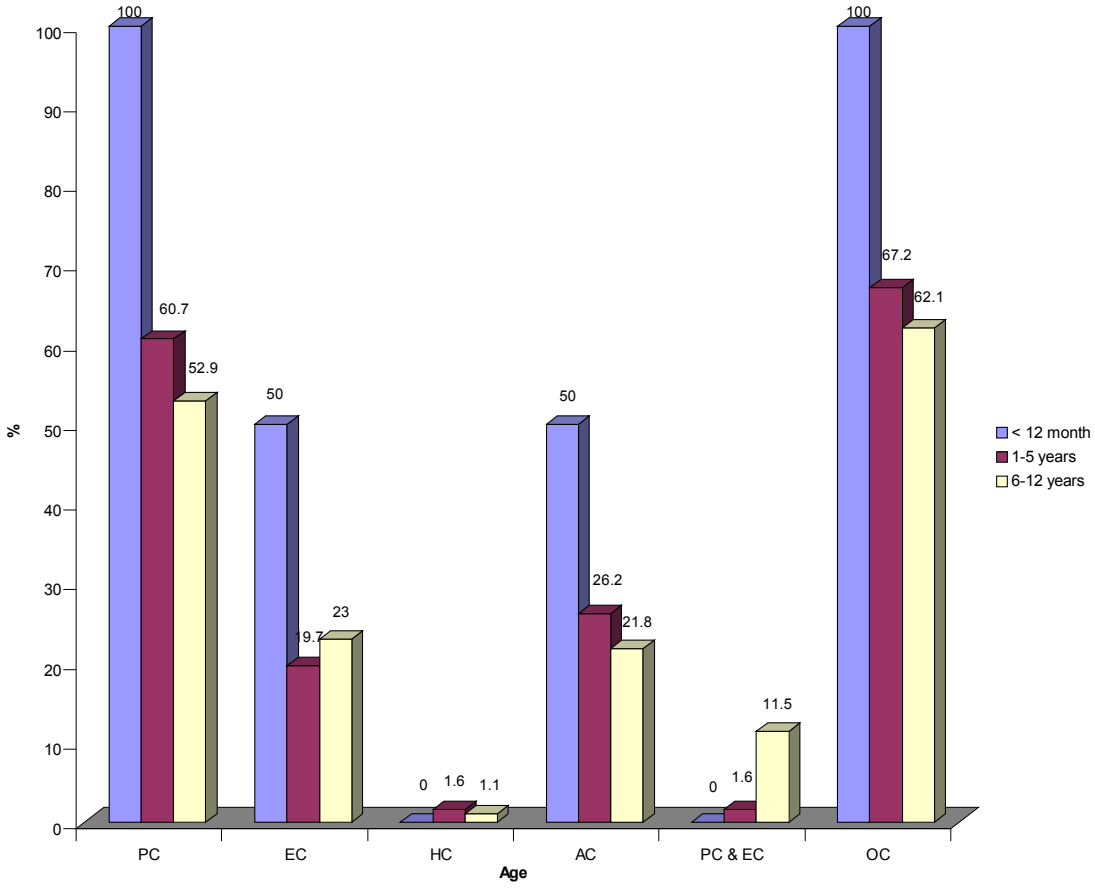


**Table 7: HIV infected pediatric patients - Clinical candida type by age (n=150)**

| Clinical Candidal Type | Age | | | | | | | | p value |
|---|---|---|---|---|---|---|---|---|---|
| | <12 month | | 1-5 years | | 6-12 years | | Total | | |
| | n | % | n | % | n | % | n | % | |
| PC | 2 | 100 | 37 | 60.7 | 46 | 52.9 | 85 | 56.7 | 0.29 |
| EC | 1 | 50 | 12 | 19.7 | 20 | 23 | 33 | 22 | 0.56 |
| HC | 0 | 0 | 1 | 1.6 | 1 | 1.1 | 2 | 1.3 | 0.95 |
| AC | 1 | 50 | 16 | 26.2 | 19 | 21.8 | 36 | 24 | 0.56 |
| PC & EC | 0 | 0 | 1 | 1.6 | 10 | 11.5 | 11 | 7.3 | 0.71 |
| OC | 2 | 100 | 41 | 67.2 | 54 | 62.1 | 97 | 64.7 | 0.46 |

**Graph 13: HIV infected pediatric patients - Clinical candida type by age**



**Isolation and speciation of oral candida in HIV infected pediatric patients (n=122)**

**Table 8: Isolation and speciation of oral candida - Clinical candida status by gender**

| Candidia | Male n=66 | (%) | Female n=56 | (%) | P value | Total n=122 | (%) |
|---|---|---|---|---|---|---|---|
| Asymptomatic | 20 | 30.3 | 16 | 28.6 | 0.497 | 36 | 29.5 |
| Symptomatic | 46 | 69.7 | 40 | 71.4 | | 86 | 70.5 |

**Graph 14: Isolation and speciation of oral candida - clinical candida status by gender**

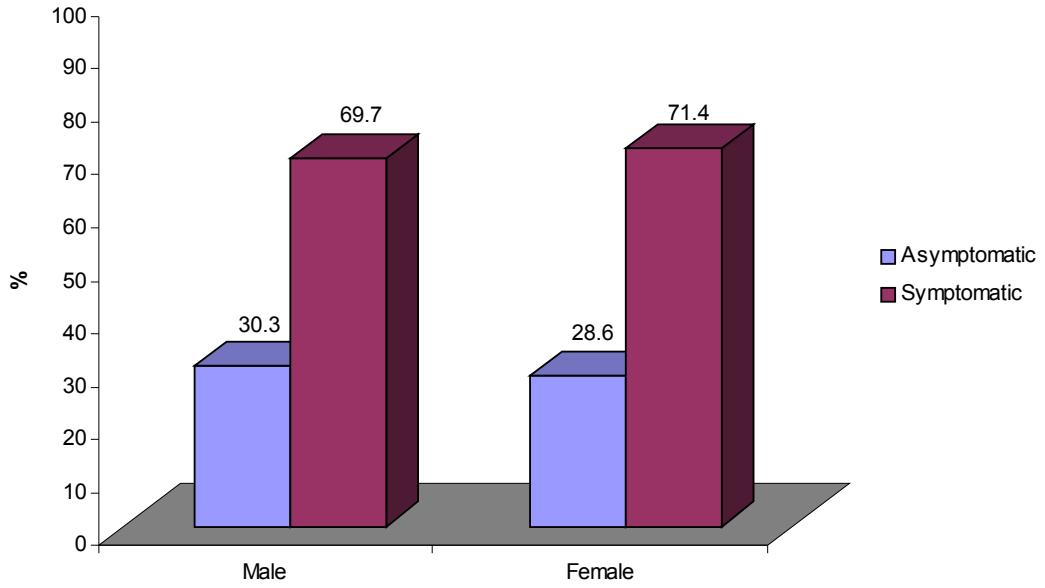


**Table 9: Isolation and speciation of oral candida - Candida species by gender (n=122)**

| Species / Gender | Male n=66 | (%) | Female n=56 | (%) | P value | Total n=122 | (%) |
|---|---|---|---|---|---|---|---|
| C.albicans | 17 | 25.8 | 15 | 26.8 | | 32 | 26.2 |
| C.tropicalis | 11 | 16.7 | 13 | 23.2 | | 24 | 19.7 |
| C.krusei | 4 | 6.1 | 1 | 1.8 | | 5 | 4.1 |
| C.albicans + krusei | 1 | 1.5 | 1 | 1.8 | | 2 | 1.6 |
| C.albicans + tropicalis | 3 | 4.5 | 5 | 8.9 | | 8 | 6.6 |
| Others + albicans | 2 | 3.0 | 2 | 3.6 | | 4 | 3.3 |
| Others+ tropicalis | 2 | 3.0 | - | - | | 2 | 1.6 |
| C.krusei + C.tropicalis | 1 | 1.5 | 1 | 1.8 | 0.833 | 2 | 1.6 |
| No | 20 | 30.3 | 15 | 26.8 | | 35 | 28.7 |
| Others (nonspecific species) | 5 | 7.6 | 3 | 5.4 | | 8 | 6.6 |
| TOTAL | 66 | 100 | 56 | 100 | | 122 | 100 |

**Graph 15: Isolation and speciation of oral candida - candida species by gender**

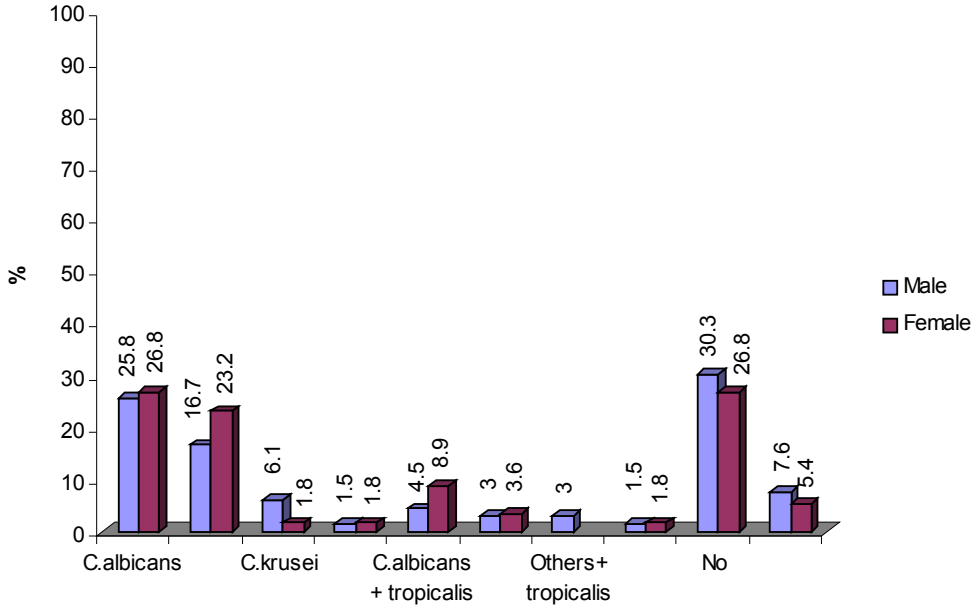



**Table 10: Isolation and speciation of oral candida - candida species by site (n=122)**

| SPECIES / SITE | Dorsum + palate | | Lateral tongue | | Hard palate | | Dorsum tongue | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % |
| C.albicans | 3 | 11.5 | 2 | 100 | 1 | 25.0 | 26 | 28.9 | 32 | 26.2 |
| C.tropicalis | 6 | 23.1 | - | - | 1 | 25.0 | 17 | 18.9 | 24 | 19.7 |
| C.krusei | - | - | - | - | - | - | 5 | 5.6 | 5 | 4.1 |
| C.albicans + krusei | 2 | 7.7 | - | - | - | - | - | - | 2 | 1.6 |
| C.albicans + tropicalis | 8 | 30.8 | - | - | - | - | - | - | 8 | 6.6 |
| Others + albicans | 4 | 15.4 | - | - | - | - | - | - | 4 | 3.3 |
| Others+ tropicalis | 1 | 3.8 | - | - | - | - | 1 | 1.1 | 2 | 1.6 |
| C.krusei + C.tropicalis | 2 | 7.7 | - | - | - | - | - | - | 2 | 1.6 |
| No | - | - | - | - | 2 | 50 | 33 | 36.7 | 35 | 28.7 |
| Others (nonspecific species) | - | - | - | - | - | - | 8 | 8.9 | 8 | 6.6 |
| TOTAL | 26 | 100 | 2 | 100 | 4 | 100 | 90 | 100 | 122 | 100 |

**P value : 0.00****

**Graph 16: Isolation and speciation of oral candida - candida species by site**

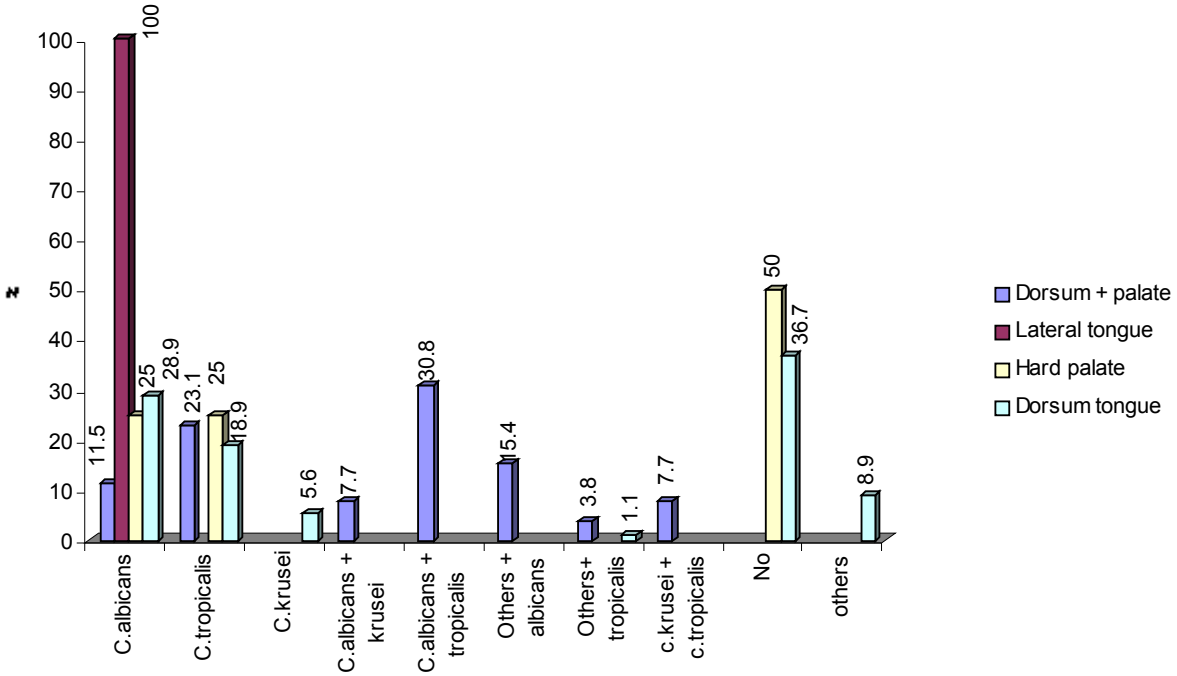



**Table 11: Isolation and speciation of oral candida - Candida species on the dorsum of tongue in symptomatic and asymptomatic group (n=122)**

| Candida species | Candidiasis | | | | Total | |
|---|---|---|---|---|---|---|
| | Asymptomatic | | symptomatic | | | |
| | n | % | n | % | | |
| C.albicans | 6 | 16.7 | 20 | 37.0 | 26 | 28.9 |
| C.tropicalis | 6 | 16.7 | 11 | 20.4 | 17 | 18.9 |
| C.krusei | 2 | 5.6 | 3 | 5.6 | 5 | 5.6 |
| Others + C.tropicalsis | 1 | 2.8 | - | - | 1 | 1.1 |
| No | 20 | 55.6 | 13 | 24.1 | 33 | 36.7 |
| Others (nonspecific species) | 1 | 2.8 | 7 | 13.0 | 8 | 8.9 |
| TOTAL | 36 | 100 | 54 | 100 | 90 | 100 |

**p value : 0.022**

**Graph 17: Isolation and speciation of oral candida- Candida species on the dorsum of tongue in symptomatic and asymptomatic group**

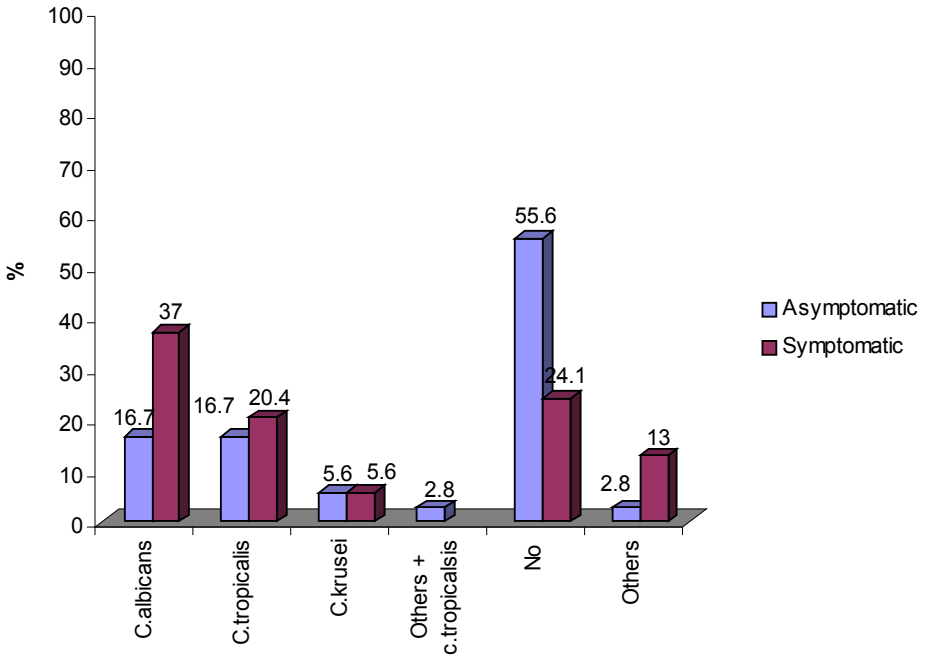



**Table 12: Isolation and speciation of oral candida - Mean CFU**

| CANDIDIA | CFU x10$^3$units/ml | Std. deviation | p value |
|---|---|---|---|
| Asymptomatic (n=26) | 6.269 | 3.06 | 0.000** |
| Symptomatic (n=82) | 16.06 | 3.17 | |

**P value: <0.01

**Graph 18: Isolation and speciation of oral candida - Mean CFU**

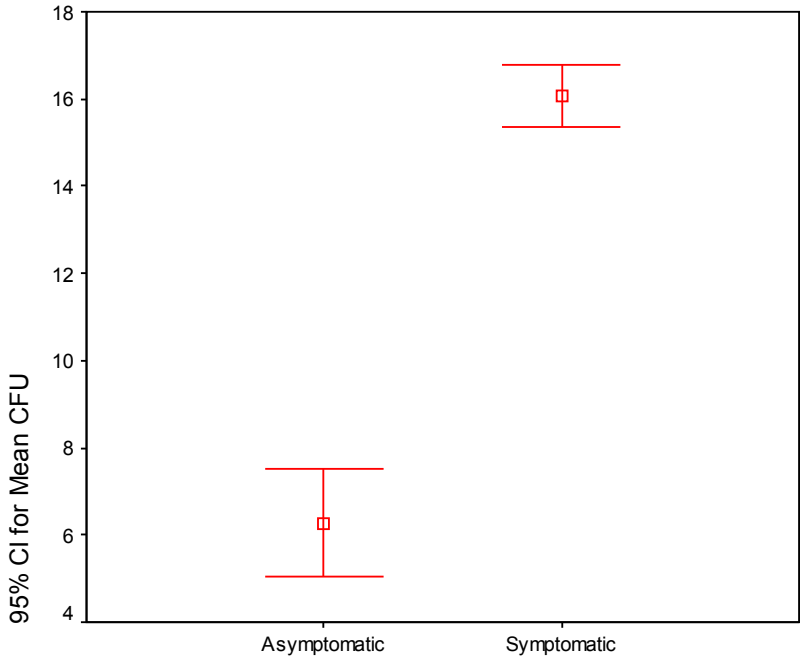


**Table 13: Isolation and speciation of oral candida - Mean CFU count by gender**

| Sex | CFU x$10^3$units/ml | Std. deviation | p value |
|---|---|---|---|
| Male (n=60) | 12.90 | 5.30 | 0.076 |
| Female (n=48) | 14.70 | 5.05 | |

**Graphs 19: Isolation and speciation of oral candida - Mean CFU count by gender**

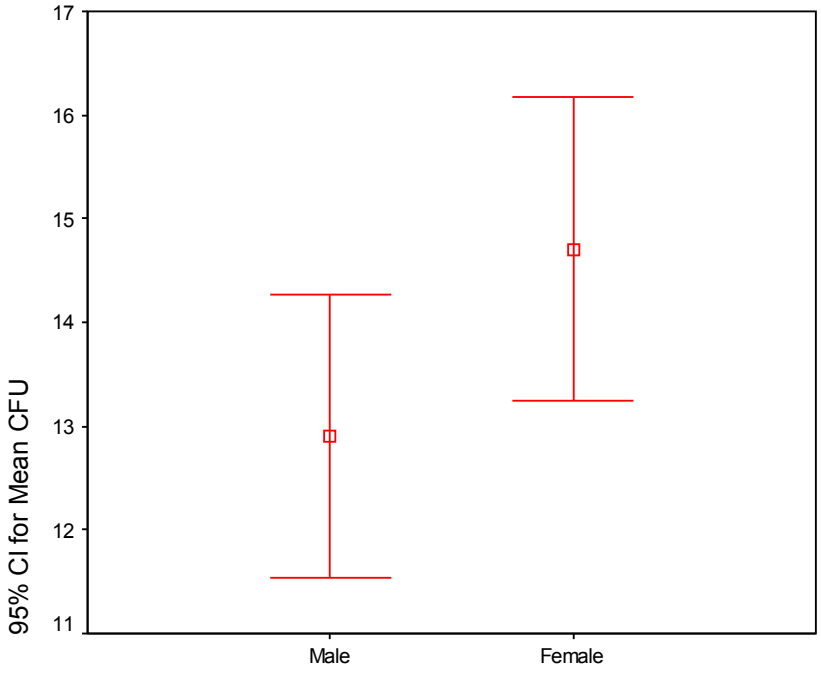


**Table 14: Isolation and speciation of oral candida - Mean CFU by clinical candida type**

| Type / CFU | n | CFU x$10^3$units/ml | Std. deviation | p value |
|---|---|---|---|---|
| PC | 40 | 16.31 | 3.09 | 0.449 |
| EC | 9 | 14.62 | 3.12 | |
| HC | 2 | 17.80 | 2.26 | |
| PC &EC | 31 | 16.05 | 3.34 | |
| **Total** | 82 | 16.06 | 3.17 | |

**Graph 20: Isolation and speciation of oral candida- Mean CFU by clinical candida type**

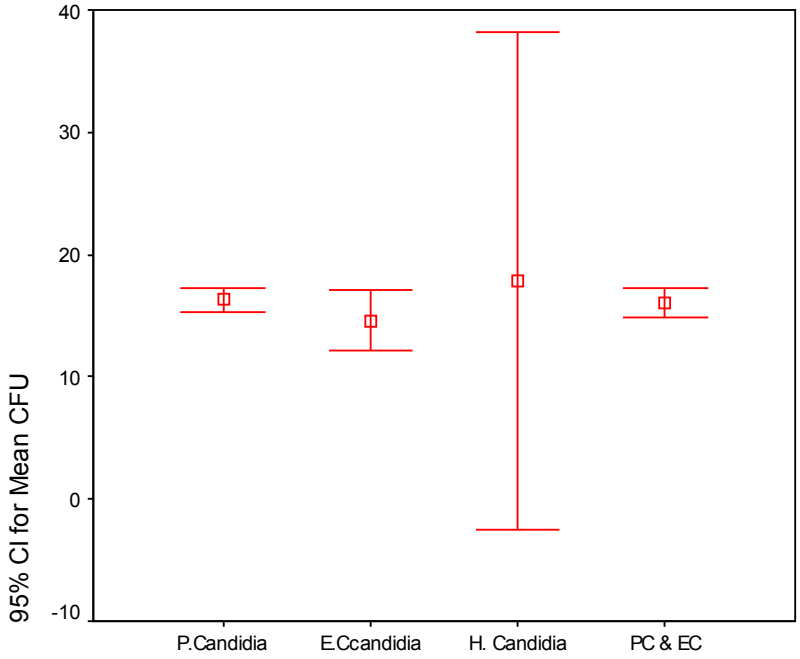


**Table 15: Isolation and speciation of oral candida - Mean CFU by immunological status (n=122)**

| CD4 | n | CFU | p value |
|---|---|---|---|
| No suppression | 17 | 6.35 | 0.241 |
| Moderate suppression | 25 | 4.21 | |
| Severe suppression | 29 | 4.81 | |

**Graph 21: Isolation and speciation of oral candida - Mean CFU by immunological status**

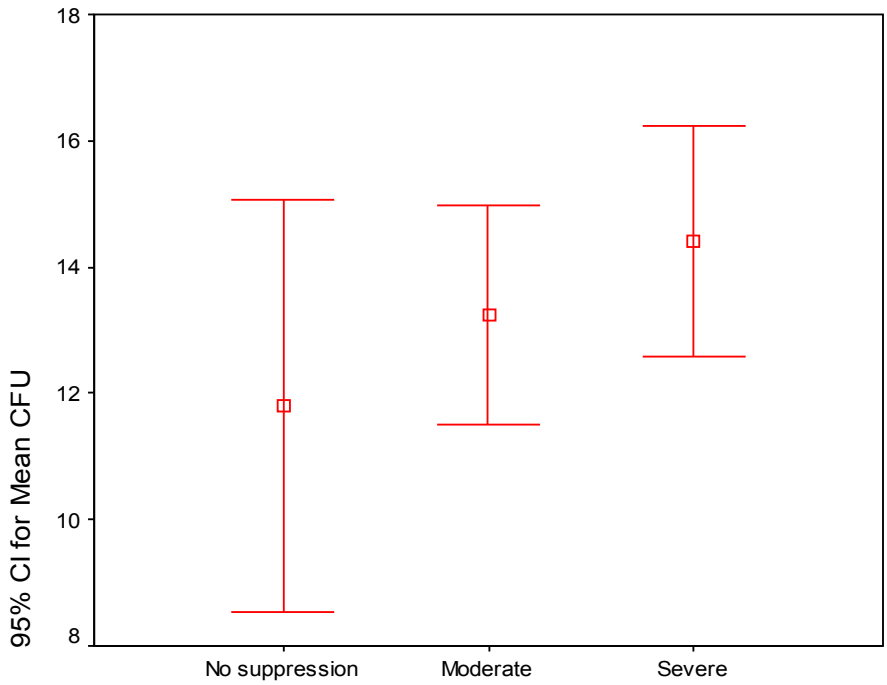
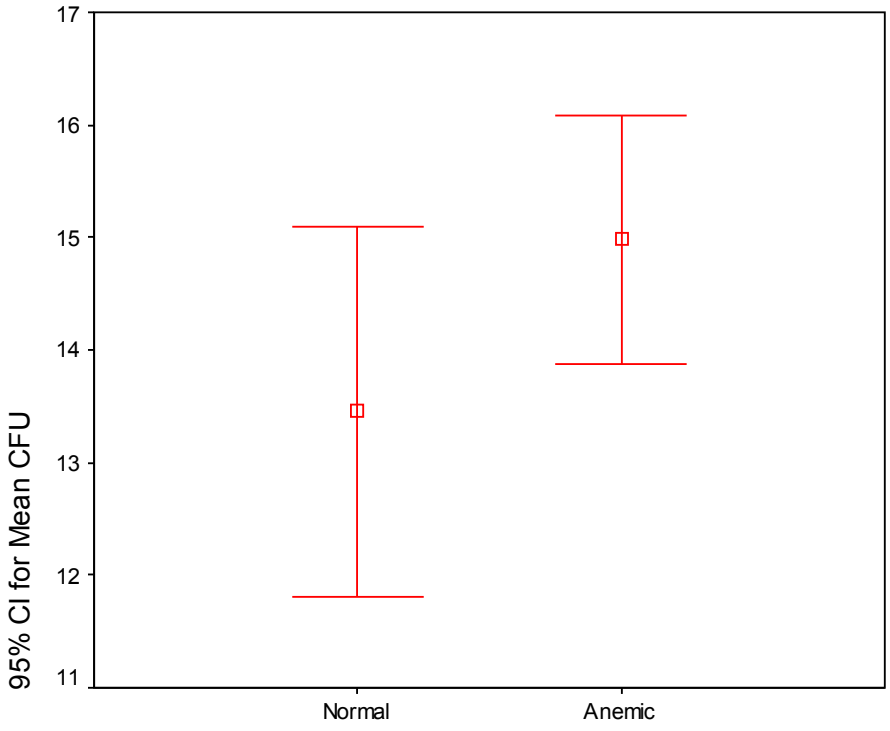


**Table 16: Isolation and speciation of oral candidiasis - Mean CFU by hemoglobin status (n=122)**

| Hemoglobin | N | Mean CFU | Std. Deviation | P value |
|---|---|---|---|---|
| Normal | 60 | 13.61 | 5.62 | 0.15 |
| Anemic* | 45 | 15.1 | 3.57 | |

*** Hemoglobin level ≤ 9mg/dl**

**Graphs 22: Isolation and speciation of oral candida - Mean CFU by hemoglobin status**




Hemoglobine level less than 9mg/dl

# *Photographs*

**Figure 1: Lymphadenopathy**

**Figure 2: Cold abscess**

**Figure 3: cold abscess**

**Figure 4: Parotid gland enlargement**

**Figure 5: Impetigo**

**Figure 6: Scabies**

**Figure 7: Oropharyngeal candidiasis**

**Figure 8: Herpetic eruptions**

**Figure 9: Herpes zoster**

**Figure 10: Herpes zoster**

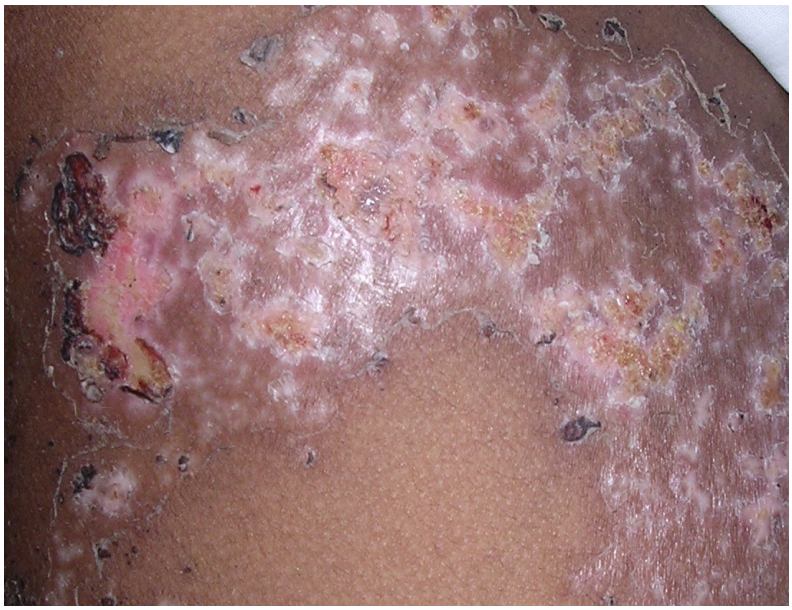


**Figure 11: Molluscum Contagiosum**

**Figure 12: Puritic Papillary Eruption**

**Figure 13: Pseudomembranous candidiasis**

**Figure 14: Pseudomembranous Candidiasis**

**Figure 15: Erythematous candidiasis**

**Figure 16: Hyperplastic Candidiasis**

**Figure 17: Angular Chelitis**

**Figure 18: Pigmentation**

**Figure 19: Pigmentation**

**Figure 20: Oral Hairy leukoplakia**

**Figure 21: Central depapillation of tongue**

**Figure 22: Complete depapillation of tongue**

**Figure 23 : Dental caries**

**Figure 25 : Candidal colonies on SDA agar**

**Figure 26: Subculture of candida in SDA agar**

**Figure 27: Candidal hypha-methyl violet stain**

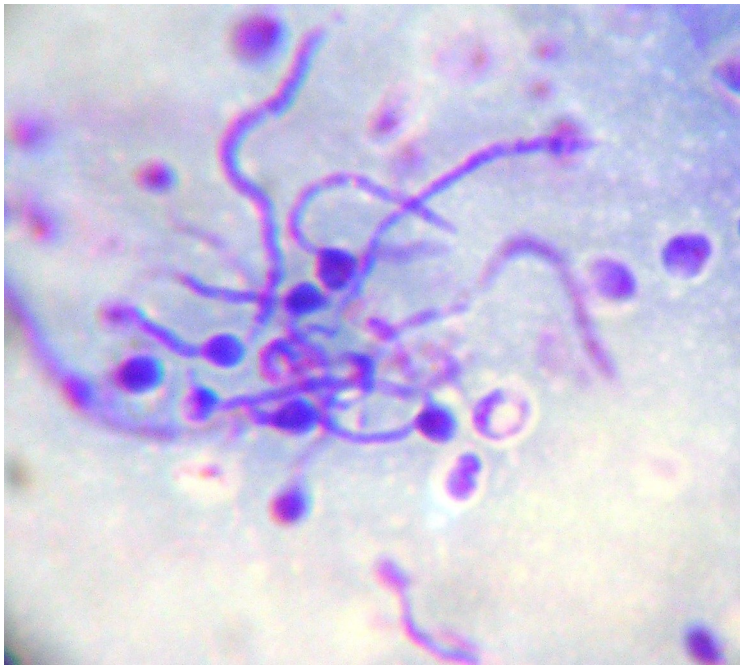


**Figure 28: Germ tube test – candidal Hypha x oil immersion**

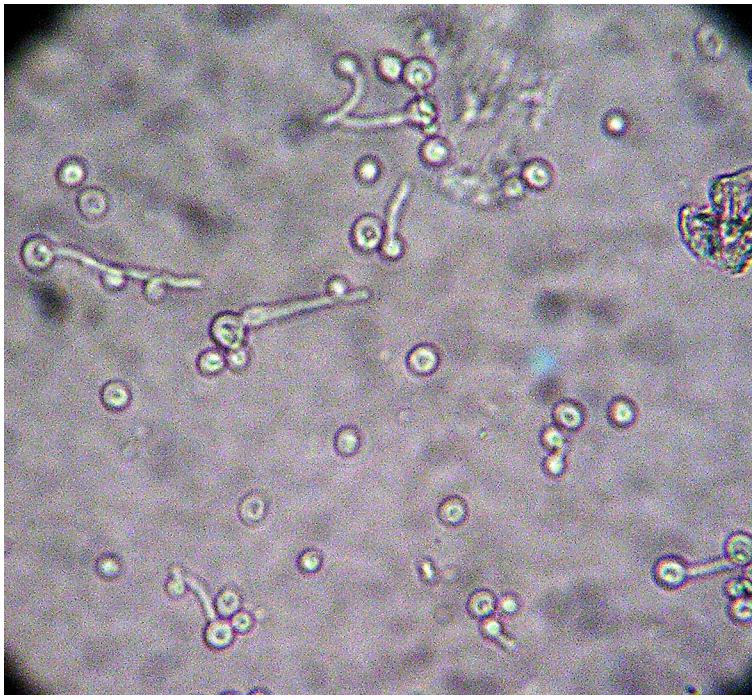


**Figure 29: Candidal species – CHROMagar**

**Figure 30: Candidal species - CHROMagar**

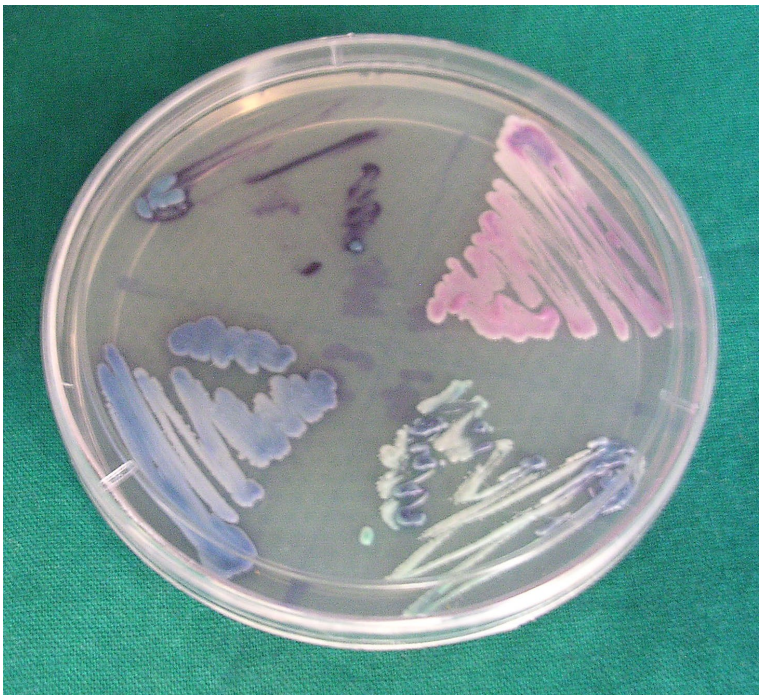


**Figure 30: Candidal species – CHROMagar**

**Figure 31: Candidal colonies on Cornmeal agar**

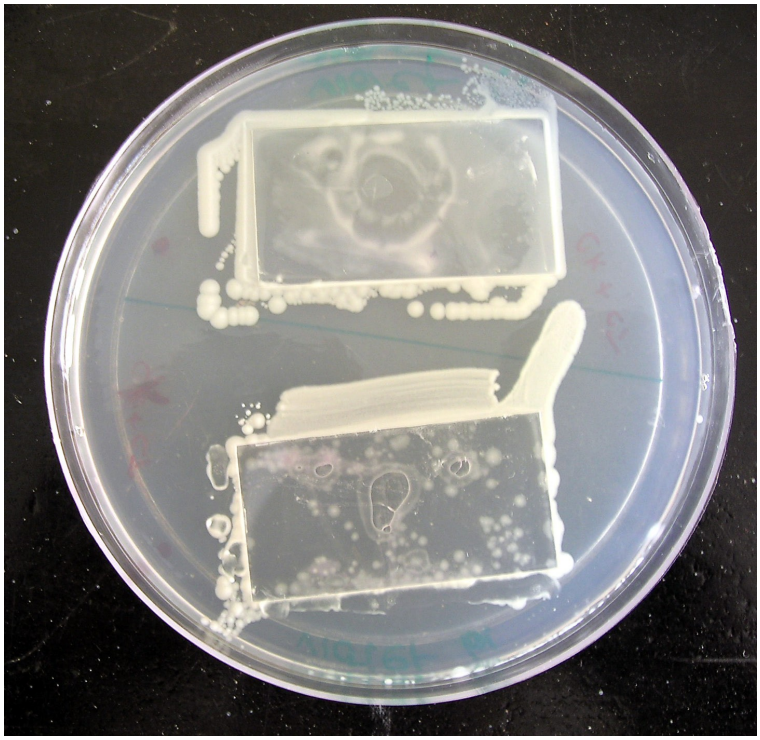


**Figure 32: Cornmeal agar- C. parapillosis 40x**

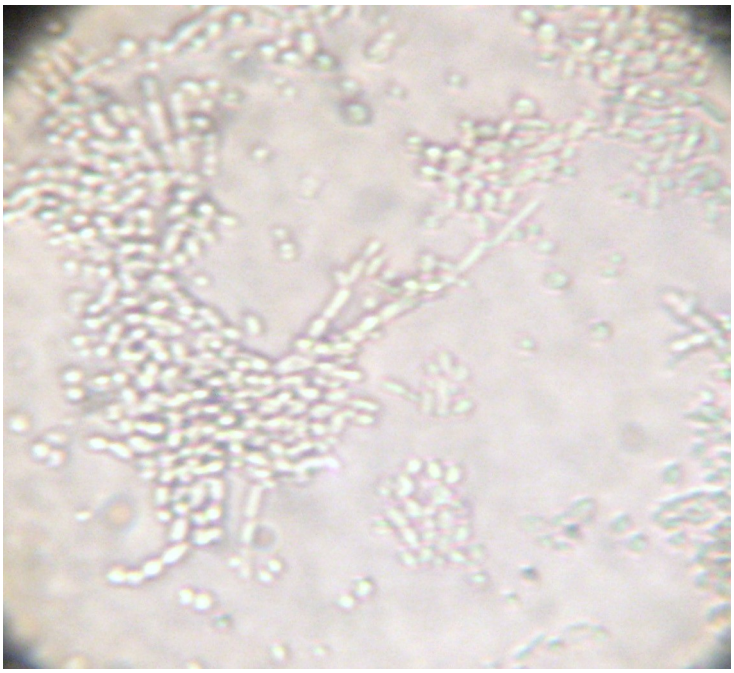


**Figure 33: Cornmeal agar- C. albicans 10x**

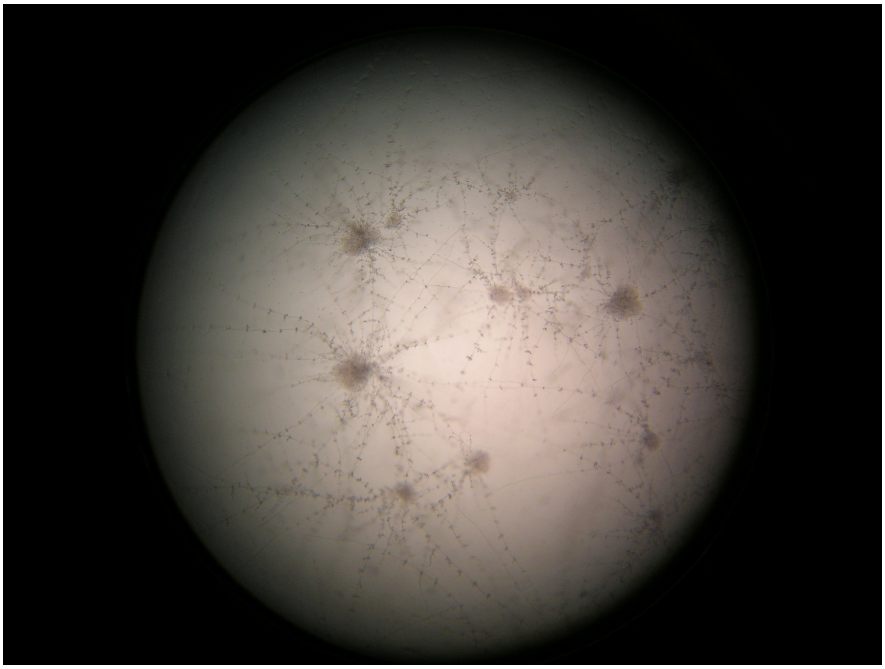
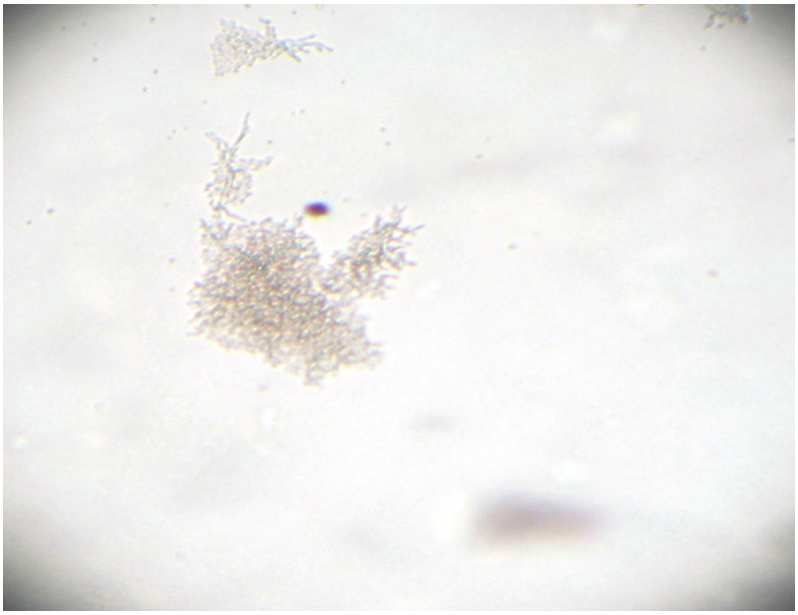


**Figure 34: Cornmeal agar- C. tropicalis 10x**



# *Discussion*

HIV infection and AIDS are spreading rapidly among the pediatric population since the report of the first case in 1982[57]. Over 20 million people have died from AIDS since the first case was identified in 1981. Today, about 37.8 million people (range: 34.6-42.3 million) are living with HIV; 2.1 million (range: 1.9-2.5 million) of them are children under the age of 15. More than 1,900 children worldwide are infected with HIV each day. In 2005 of 2.9 million people (range: 2.6-3.3 million) who died of AIDS, 490,000 (range: 440,000-580,000) were children. This amounts to approximately 1,350 AIDS deaths in children per day, worldwide. World wide 12 million children have lost one or both parents to AIDS. By 2010, this number is expected to rise to more than 18 million. In the developed countries there are only a few hundred or a few thousand orphans but in the developing countries there are millions and this difference is due to the accessibility to ART in the developed countries. In India the estimated number of children infected with HIV infection is 120,000[138].

Oral lesions are common in adults with HIV infection and have diagnostic and prognostic significance[109]. Although oral manifestations of HIV infection in adults have been well established, little is known about oral lesions in children, especially Indian children.

In this study we studied systemic and oral manifestations in 150 children infected with HIV. The male and female ration was 1.7:1. This is similar to the reports by **Lucas SB et al 1996**[76] in their study of 78 pediatric patients in Abidjan population and **Matida LH et al 2004**[86] in their study of 914 pediatric patients in Brazil.

In our study, though there was no significant difference in distribution of children

in different age groups, more than 50% were in the 6-12 year age group. This finding was similar to the reports of **Kozinetz CA et al 2001**[71] from 62 patients in Romania and **Agarwal M et al 2001**[2] on 821 patients in India, and observed that the prevalence of HIV infection in this cohort was more in children between the age group of 7-12 years.

HIV can be transmitted perinatally from a mother to her new born infant (vertical transmission) in three ways: (1) transplacentally during pregnancy; (2) during delivery, as the infant passes through the birth canal (estimated to be 40% of the cases); or (3) postnatal, during breast feeding. Ninety four percent of pediatric HIV infections are acquired perinatally, and the vast majority are acquired during delivery[45, 48]. In developing countries majority of children are infected with HIV either in antepartum (20% before childbirth); intrapartum (40% during childbirth), or breastfeeding (40%) [138].

.In our study the predominant mode of acquiring HIV infection was by vertical transmission during the time of delivery and this finding was consistent with that of **Hermione EG et al in 2002**[53] who reported that 70% of their patients acquired HIV infection through vertical transmission. **Merchant RH et al 2001** [87] reported vertical transmission in 247 (86.99%) cases and stated that vertical transmission was the major mode of HIV infection seen in India.

Breast feeding is the second major mode of vertical transmission seen among the pediatric group in the developing countries [13, 45] In our study 81 (54%) children were breast fed and the mean duration was 2.5years from the time of birth. It has been reported that the overall risk of transmission of HIV from mother to child is 15-25% for non breast-feeding mother, and 25-45%, for breast-feeding mother[53]. In our study, also breast-feeding may have contributed to a mode of vertical transmission.

Infection with HIV results in profound immunosuppression rendering the host susceptible to various infections and neoplasms[91]. In non - infected children the CD4 cell count is higher than in the adults, but declines over the first 6 years of life to approach adult levels, by the age of 6 years [45.] The disease progression in HIV infected child is rapid and fulminating since most of the immune system is still in the developing stage[2].

Based on the immune categories, age of the children and clinical categories, CDC has classified the children with HIV infection, into three categories . In our study 25% were in category I (No suppression), 39.4% were in category 2 (moderate suppression) and 36.2% were in category 3 (severe immune suppression).

Clinical features of HIV infection are common to both adults and children, but some are more typical and pronounced among the pediatric population [45]. Failure to thrive, which is associated with an abnormal growth rate, may affect as many as 50% [45] to 95% [119, 75.] Tuberculosis[2, 8, 75, 92] and generalized lymphadenopathy[8,2,71,119]are the common systemic lesions seen in pediatric children infected with HIV in the developing world. Other conditions more commonly found among children are lymphoid interstitial pneumonia[45,92] invasive and recurrent bacterial infections[45,86,92,102], pnemocystis carinii pneumonia[76,86,102], respiratory tract infection[71,75,76], otitis media[71], bacterial septicemias[102], parotitis[45,] CMV[102], meningitis[86], chronic diarrhea[2,8], persistent cough[2,8], severe malnutrition[2,8], generalized dermatitis[2,8,118], disseminated herpes simplex[92], splenomegaly[118], hepatomeglay[2,8,71] and neoplasms namely lymphoma[45,92] and leiomyosarcoma[45].

In this study all the 150 patients had one or more systemic lesions, the most common lesion observed in our study was lymphadenopathy in 125 patients (83.3%).

Similar to reports by **Spira R et al 1999[119] (Rwanda)** in 347 children, **Naidoo S et al 2004[91] (South Africa)** in 169 children and **Kozinetz et al in 200l[70] (Brazil)** in 73 children, showed lymphadenopathy as the most common lesion in 91.1%, 92% and 51% respectively.

In our study 61% of the children presented with tuberculosis (TB) and this include both pulmonary tuberculosis (PTB, 21.3%), primary complex 25.3% and extra pulmonary tuberculosis (ETB 45.4%). **Swaminathan S 2004[122] (chennai),** in his study of 87 patients reported disseminated TB in 34% and pulmonary TB in 37% of cases**. Lodha R et al 2000 [75] (New Delhi),** reported disseminated TB in 27.3% of patients in their study of 27 HIV patients. **Bavdekar SB et al 2005[8] (Mumbai)** observed disseminated TB in 22.7% of cases out of 115 patients**, Kozinetz et al in 200l[71] (Romania)** and **Palme IB et al in 2001[96] (Ethiopia)** reported that 11.2% of children in their study had pulmonary tuberculosis. In the study done by **Jones DS et al in 1997[71] (New Jersey)** and **Chan SP et al in 1996 [26] (New York),** the prevalence of PTB was 21% and 55% respectively. In our study extra pulmonary variants of tuberculosis observed were cold abscess in 45.4% of patients. There is no other similar report to our knowledge from the developing countries, which has detailed the occurrence of variants of extra pulmonary TB. In our study we also observed that there was a significant difference in the occurrence of TB between gender.

Measles was the next common systemic lesion in our study and it was seen in more than 50% of the children. Similar prevalence rate have been reported by **Lucas SB et al 1996[76] (Abidjan)** in 19% of pediatric patients and was more common in children greater than 15 months of age in their study.

Other opportunistic infections seen in our study were otitis media in 48.7% of cases, upper respiratory tract infection (URTI) in 36% of cases and mumps in 24.7% of cases, **Kozinetz et al in 200l[71](Romania)** have reported otitis media in 97% of 762 HIV infected children.

In our cohort study the cutaneous lesions observed were scabies (15.3%), impetigo (2.7%) and pruritic eruption in 52.1% of cases. **Bavdekar et al 2005[8] (Mumbai)** in their study of 115 pediatric patients have reported generalized dermatitis which also included pruitic eruption and eczema in 83.3% of patients. There was only one case of molluscum contagiosum in our study and this entity was also reported by **Faitz C et al 2000[36](Romania)** in 3% of 173 pediatric patients and by **Merchant RH et al 2001(India)[87]** in 3 cases out of 285 patients in their study.

Parotid gland enlargement may be caused by infiltration of CD8 cells which are cytotoxic to HIV infected cells and have the ability to destroy the virus, probably explaining the slower progression in children with such pathology[45]. In our study we found 18.7% of patients had parotid gland enlargement. Parotid gland enlargement was also reported by **Dos LD et al 2000[30]** and **Luciane RR etal 1998[77]** in Brazil, but the prevalence was 9% and 7.3% respectively. Other authors have also reported parotid gland enlargement in their study [36, 70, 71,106,126]

Herpetic eruption of the perioral areas was observed in 2 patients (1.3%) in our study and this finding was similar to that reported by **Dos LD et al 2000[30] ( Brazil)** in their study of 80 pediatric patients and **Romos F et al 1996[107] (USA)** in their study of 492 pediatric patients.

In our study, 4 (18.7%) children had perioral warts. **Flatiz et al 2000[36] (Romania)** observed oral warts in 2% of 172 pediatric patients in their study and one case has been reported by **Magalhaes et al 2001[81](Brazil)** in their study of 38 pediatric HIV infected patients. To our knowledge this is the first report from in India , which of oral warts in HIV infected pediatric patients.

Systemic candidiasis was seen in 2 cases (1.3%). Oropharyngeal candidiasis and vaginal candidiasis were seen in 4 cases each. The 4 patients with oropharyngeal candidiasis developed dysphagia. Our clinical observation of dysphagia in patients with oropharyngeal candidiasis was consistent with that reported by **EiHachem et al 1998[32] (Brazil)** in their study of 85 HIV infected Brazilian children.

Oral mucosal lesions are one of the earliest clinical indicators of HIV infection and progression in children[48]**.** Presentations of orofacial lesion in patients with HIV infection are strongly associated with immune suppression [48]. The occurrence of oral lesions is probably geographically site-specific, because the frequency of specific oral lesions differ regionally.[48]

In our study the most common oral lesion was oral candidiasis, seen in 64.7% of the patients and this finding was similar to the reports of **Romos F et al 1996[107] (California).** In their study of 495 patients, where they observed oral candidiasis in 67% of cases and **Naidoo S et al 2004[91] (South Africa)** in their study of 169 patients who reported oral candidiasis in 63% of their cohort. **Lodha R et al 2000[75] (India)** in their study of 27 HIV infected children observed OC in 36.4 % of cases and the low prevalence of oral candidiasis in their study was due to small sample size.

Of the variants of OC in our study PC was observed in 56.7% of children and this was the predominant clinical type of candidiasis. This finding was similar to the findings of **Naidoo S et al 2004[91](South Africa)** and **Romos F et al 1996[107] (California)** who reported 50% and 47% of PC in their respective studies. Other authors have also reported oral candidiasis as the most common lesion in their studies [8,30, 47,55, 70, 71,75,77, 100,108,126].

The prevalence of AC (24%) in our study was slightly higher compared to the studies reported by **Naidoo S 2004[91](South Africa), Luciane RR et al 1998[77] (Brazil) and Pongsiriwes et al 2003 [102](Thailand)** and this was due to malnourishment which was prevalent in the lower socioeconomic status of our cohort.

EC was observed in 22% of our patients, and this finding was similar to reports of **Naidoo S 2004[91](South Africa),** who reported EC in 20% of their pediatric seropositive patients. **Luciane RR et al 1998[77] (Brazil), Pongsiriwes et al 2003 [102](Thailand), Barash A et al 2000[5] (Roman) are** few others who reported low prevalence of EC compared to our study and this was due to smaller sample size in their study.

We observed HC in 2 cases and this finding was similar to that of **Naidoo S 2004[91] and Flanagan et al 2000[39]** who also reported HC in their study group.

Combined lesions of PC and EC was observed in 7.3% of cases in our study and this finding was consistent with that of **Flanagan et al 2000[44] (New Jersey)**.

Next common oral lesion in our study group was conventional gingivitis, observed in 9.3%, of the patients, we did not come across linear gingival erythema. Conventional gingivitis has also been reported by **Barash A et al 2000[5](Roman), Luciane RR et al 1998[77] (Brazil), Gomez FJ et al 2000 [47] (USA) Schoen DN et al 2000[117] (USA) and Flaitz C et al 2000[36] Romania)** and the prevalence is similar to that

of our study. **Howell RB 1996[57] (USA),Gelbier M et al 2000[44] (London) and Carvalho L et al 2005[30] (Brazil)** not only reported a high prevalence of conventional gingivitis, but also found an association between conventional gingivitis and immunosuppression.

In our study we observed OHL in 5.3% of patients. **Flaitz C et al 2000[36](Romania)** in their study of 173 patients have reported OHL in 2% of their patients. **Naidoo S 2004[91]** (**South Africa)** reported OHL in 1% of 169 pediatric patients. **Barash A et al 2000[5] (USA) and Kozinetz C et al 2000[70] (USA)** have reported OHL in 2% of their study group**. Pongsiriwes et al 2003 [102](Thailand)** observed OHL in 22.5% of cases in their study. This high prevalence of OHL in their study was due to the fact that OHL is widely prevalent in Thailand, due to high prevalence of EB virus.

Kaposi's sarcoma is the most common neoplasm developing among HIV infected adults and is rare in children[45]. In our study we observed Kaposi's sarcoma of the palate in one case. **Kozinetz C et al 2001[71] (Brazil)** had a similar observation.

One of the unique finding in our study was the oral pigmentation present in 12 patients (8%). Pigmentation was seen on the dorsal surface of tongue, hard palate and on the buccal mucosa. In our study, children who presented with oral pigmentation were on iron supplements. The existing anemic in these children could have been the contributing factor to oral pigmentation in these children.

6% of patients had depapillation of the dorsum of tongue, and these lesion were positive for candida albicans. **Flanagan et al 2000[39](New Jersy)** observed similar lesion in 8% of pediatric patient cases in their study of 37 HIV infected pediatric patients.

In our study we observed oral ulcers in 8 patients (5.3%) which were clinically suggestive of apthous ulcers and was seen predominantly on the buccal mucosa, This

finding was similar to the reports of **Toro AD et al 1996[127](New York) and Luciane RR et al 1998[77] (Brazil)** who reported oral ulcers in 5% of their cases.

Candida albicans is a normal commensal of the oral cavity. The normal candidal carriage rate in healthy adult ranges from 3% to 48% and in healthy children it is 45% to 65% [88]. Lowest candidal carriage rates are seen in neonates which is estimated to be 9% to 16%[78].

In our study we attempted to isolate and identify the candida species in 122 children, with and without clinical candidiasis referred to as symptomatic and asymptomatic respectively. Of the 122 patients 86 (70.5%) patients, were found with a diagnosis with clinical candidiasis (symptomatic for candida) . Of these 86 symptomatic patients, we were unable to isolate candida from 13 patients. When we analyzed these results, we found that these 13 patients had a clinical diagnosis of EC, **Reichart PA et al in 2000[111]** in their study explained that, in their experience, cases which presented as EC clinically when subjected to laboratory investigation did not show any growth on the primary the culture medium. This could be due to the fact that EC possess only blastopore, which is difficult to grow in the laboratory.

In our study there were 36 patients who were asymptomatic for candidiasis clinically. **Bosco et al 2003[15] (Brazil)** in their study of 30 HIV infected patient stated that there is an association between the level of immune suppression and candidiasis. **Hicks JM et al in 1998[54] (London)** in 27 HIV pediatric patients found that yeast and fungal organisms were isolated more from patients with severe immune suppression state. We were able to isolate candida in 16 patients and in 20 patients we could not demonstrate

the growth. The possible reason of this could be due to the fact that most of the patients, in whom candida could not be isolated, had high CD4 count.

Candida was speciated by CHROMagar technique[93]. The common species isolated were C.albicans, C.tropicalis, C.krusei, mixed colonies of C.albicans with C.krusei and C.albicans with C.tropicalis. Other species (which could not be typed with CHROM agar) and mixed colonies of other species with C.albicans & with C.tropicalis were also speciated. In our study, C.albicans was the predominant species isolated, followed by C.tropicalis and this finding was consistent with that of **Bosco VL et al in 2003[15] (Brazil)** who isolated C.albicans as predominant species in 23% patients. Mixed colonies of C.albicans with C.topicalis & C.krusie were the next prevalent combination of candidal species isolated in our study. There was an equal prevalence of mixed colonies of other species with C.albicans and with C.tropicalis.

8 cases of non specific species which was isolated by CHROMagar in our study were identified as C.parapsilosis in two cases, mixed colonies of C.tropicalis with C.parapsilosis in 6 samples with cornmeal agar. **Bosco VL et al in 2003[15] (Brazil)** isolated C.parapsilosis in 30 HIV positive pediatric patients using sugar assimilation test. **Kim JO et al 2003[66] (Philadelphia)** isolated C.dubliniensis from 205 patients using API 20C system, this finding illustrates that CHROMagar technique is not sensitive to subtype all candidal species[10,93].

Of the 90 children for whom the samples were collected from the dorsum of tongue, hard palate and the lateral surface of tongue. C.albicans was the most predominant species isolated in 29% of them and the next prevalent species isolated was C.tropicalis in 19.0% of the patients.

In our study, in both symptomatic and asymptomatic HIV positive pediatric children the predominant species isolated was C.albicans and C.tropicalis. There was a significantly lower prevalence of candida in asymptomatic than in symptomatic patients.

CFU count of candida in asymptomatic HIV infected adult patients was estimated to be $<4 \times 10^3$ units/ml and for symptomatic patients it is $>4 \times 10^3$ units/ml [111.] To our knowledge CFU count of candida is not established in HIV infected children.

In our study the CFU count of candida was calculated for all the samples as described by **Samarnayaka et al 1990.** In our study CFU count in asymptomatic patients was $6.3 \times 10^3$ CFU /ml and in symptomatic patients was $16.0 \times 10^3$ CFU /ml and there was a statistically significant difference in CFU count between the two groups. The CFU count in our pediatric population appears to be high when compared to the CFU count in adult this difference may be due to the immature immune system seen in pediatric population.

**Bosco et al 2003[15](Brazil)** in 30 children observed that CFU count was high in severely immunosupressed patient compared to children with lower degree of immunosuppression. Though we observed a difference in the CFU count of candida between the children with varying degree of immunosupression, the difference was not statistically significant.

# *Summary & Conclusion*

- A total of 150 HIV seropositive pediatric patients were screened for oral and systemic lesions. Candidal samples were collected for 122 patients using swab technique and CFU was estimated. Candidal species were isolated by using CHROMagar.
- Out of 150 patients, 82 (55.7%) were males and 68 (45.3%) were females and majority of them were between the age group of 6-12 years.
- 139 patients (92.7%) had full term birth status and about 9(6%) patients had history of premature birth status and for the rest of the patients birth status was not known .
- The most common route of transmission in our study was vertical transmission which was seen in 143 patients (95.3%) and only 6 (4%) patients acquired HIV infection through blood transmission.
- 54% of patients were breast fed while bottle feeding was seen in 8% of patient and rest of the patients had combination of both.
- The most common systemic lesion observed in our study was lymphadenopathy followed by tuberculosis, pulmonary tuberculosis was seen in 21.3% and extra pulmonary tuberculosis was seen in 45.4% of cases. The latter manifested in the form of Cold abscess.
- Measles, otititis media, mumps and parotid gland enlargement were the other systemic infections observed in our study group. Oral pharyngeal candidiais and Systemic candidiasis was observed in 4 (2.7%) patients.
- The common skin lesions seen in our study were scabies, impetigo, herpetic eruption, perioral wart and molluscum contagiosum.

- All the 150 HIV infected children had at least one systemic and oral lesion at the time of examination.
- The common oral lesion observed was Oral candidiasis of which PC was the most predominant, followed by AC, EC, HC and combination of PC and EC.
- Conventional gingivitis, pigmentation, depapillation of tongue, oral hairy leucoplakia, oral ulcer patients and Kaposi's sarcoma were other oral lesions observed in our study.
- Samples for candida were collected from 36 asymptomatic and 86 symptomatic patients by using swab technique
- CHROMagar was used to identify the candial species. C.albicans was the common species isolated from both symptomatic and asymptomatic patients. The prevelance of Candida species is more in symptomatic patients than the asymptomatic patients which was statistical significant.
- C.tropicalis, mixed type of colonies of C.albicans with C.krusei and C.tropicalis and non-specific species with C.albicans and C.tropicalis were also identified.
- CFU count was calculated using Samarnayaka technique. The mean CFU was increased in symptomatic than in asymptomatic patients and was statistical significant.

In conclusion, all the children in this study presented with at least one oral lesion. We state that systemic and oral mucosal lesions are a feature of HIV infection in the pediatric population. Furthermore, additional studies investigating the association between oral lesion and CD4 counts in a large sample size would be beneficial for developing prognostic markers for HIV infected children in our country.

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*Annexure*

# Annexure I

## 1994 Revised classification system for pediatric HIV disease based on clinical manifestations

**Category N :** *Not symptomatic*

No signs or symptoms as a result of HIV infection or only one of the conditions listed in category A

**Category A :** *Mildly symptomatic*

Children with two or more of the following conditions but none of the conditions listed in Category B or Category

Lymphadenopathy (>0.5 cm at more than two sites or bilateral at one site)

Hepatomegaly

Splenomegaly

Dermatitis

Parotitis

Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

**Category B:** *Moderately symptomatic*

Children who have symptomatic conditions , other than those listed in for category A or Category C, that are attributed to HIV infection

Anemia (8g/dl), neutropenia (1000 cella/$mm^3$), or thrombocytopenia (<100,000cells/$mm^3$) persisting for 30 days or longer

Bacterial meningitis, pneumonia, or sepsis (single episode), candidiasis, oropharyngeal persisting for more than 2 months in children older than 6 months

Cardiomyopathy

Cytomegalovirus infection with onset before age 1 month

Diarrhea, recurrent or chronic

Hepatitis

Herpes simplex virus (HSV) stomatitis, recurrent (>two episodes within 1 year)

HSV bronchitis, pneumonitis, or esophagitis with onset before the age 1 month

Herpes zoster infection involving at least two distinct periods or more

Leiomyosarcoma

Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex

Nephropathy

Nocardiosis

Fever lasting less than 1 month

Toxoplasmosis with onset before age 1 month

Varicella, disseminated

**Category C:** *Severely symptomatic*

Children who have any condition listed in the 1987 surveillance case definition for AIDS with the exception of LIP

## Annexure II

**1994 Revised classification system for pediatric HIV disease based on CD4 cell count and percentage (CDC)**

| **Immune Category** | **<12 momths** | | **1-5 years** | | **6-12 years** | |
|---|---|---|---|---|---|---|
| | CD4 cells/$mm^3$ | % | CD4 cells/$mm^3$ | % | CD4 cells/$mm^3$ | % |
| **Category 1: No suppression** | >1500 | 25 | >1000 | >25 | >500 | >25 |
| **Category 2** **Moderate suppression** | 750-1499 | 15-24 | 500-999 | 15-24 | 200-499 | 15-24 |
| **Category 3:** *Severe suppression* | <750 | <15 | <500 | <15 | <200 | <15 |

# Annexure – III

**Classification and diagnostic criteria for oral lesions in HIV infection**

**EC Clearinghouse**

Group 1: Lesions strongly associated with HIV infection

- Candidiasis
  - Erythematous
  - Pseudomembranous
- Hairy leukoplakia
- Kaposi's sarcoma
- Non-Hodgkin's lymphoma
- Periodontal disease
  - Linear gingival erythema
  - Necrotising (ulcerative) gingivitis
  - Necrotising (ulcerative) Periodontitis

Group 2: Lesions less commonly associated with HIV infection

- Bacterial infections
  - Mycobacterium avium-intracellulare
  - Mycobaterium tuberculosis
- Melanotic hyperpigmentation
- Necrotising (ulcerative stomatitis
- Salivary gland disease
  - Dry mouth due to decreased salivary flow rate
  - Unilateral or bilateral swelling of major salaivary glands
- Thrombocytopenic purpura
- Ulceration NOS (not otherwise specified)
- Viral infections
  - Herpes simplex virus
  - Human papillomavirus (warty-like lesions)

- Condyloma accuminatum
- Focal epithelial hyperplasia
- Verruca vulgaris

Varicella-zoster virus

- Herpes zoster
- Varicella

Group 3: Lesions seen in HIV infection

Bacterial infections

- Actinomyces israelii
- Escherichia coli
- Klebsiella pneumoniae

Cat-scratch disease

Drug reactions (ulcerative, erythema multiforme, lichenoid, toxic epidermolysis)

Epithelioid (bacillary) angiomatosis

Fungal infection other than candidiasis

- Cryptococcus neoformans
- Geotrichum candidum
- Histoplasma capsulatum
- Mucoraceae (mucormycosis / zygomycosis)
- Aspergillus flaws

Neurologic disturbamces

- Facial palsy
- Trigeminal neuralgia

Recurrent aphthous stomatitis

Viral infections

- Cytomegalovirus

Mollucum contagiosum

# Annexure IV

## Classification of Orofacial Lesions Associated with Pediatric HIV infection patients using the framework of the EC-Clearinghouse and WHO (1994)

**Group 1:** Lesions commonly associated with paediatric HIV infection.

- Candidiasis
  - Erythematous
  - Pseudomembraneous
  - Angular cheilitis
- Herpes simplex virus infection
- Linear gingival erythema
- Parotid enlargement
- Recurrent aphthous ulcers
  - Minor
  - Major
  - Herpetiform

**Group 2:** Lesions less commonly associated with paediatric HIV infection.

- Bacterial infections of oral tissues
- Periodontal diseases
  - Necrotizing (ulcerative) gingivitis
  - Necrotizing (ulcerative) periodontitis
  - Necrotizing (ulcerative) stomatitis
- Seborrheic dermatitis
- Viral infections
  - Cytomegalovirus
- Human papollomavirus
- Molluscum contagiosum
- Varicella-zoster virus

Herpes-zoster

Varicella

Xerostomia

**Group 3:** Lesions strongly associated with HIV infection but rate in children.

Neoplasms

Kaposi's sarcoma and non-Hodgkin's lymphoma

Oral hairy leukoplakia

Tuberculosis-related ulcers